(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 December 2001 (06.12.2001)

PCT

(10) International Publication Number
WO 01/92218 A2

(51) International Patent Classification[7]: C07C 311/00

(21) International Application Number: PCT/US01/17795

(22) International Filing Date: 31 May 2001 (31.05.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/584,175 31 May 2000 (31.05.2000) US

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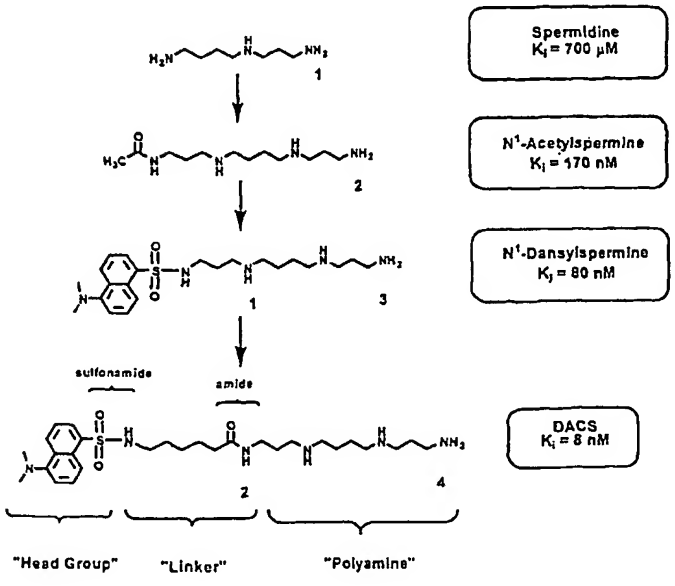
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) **Title:** NOVEL POLYAMINE ANALOGUES AS THERAPEUTIC AND DIAGNOSTIC AGENTS



(57) **Abstract:** Novel "bispolyamine" inhibitor compounds of polyamine transport are disclosed. These compounds are useful pharmaceutical agents for treating diseases where it is desired to inhibit polyamine transport or other polyamine binding proteins, for example cancer and post-angioplasty injury. These compounds display desirable activities both for diagnostic and research assays and therapy.

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NOVEL POLYAMINE ANALOGUES AS THERAPEUTIC AND DIAGNOSTIC AGENTS

FIELD OF THE INVENTION

The invention in the field of chemistry and biochemistry relates to the synthesis and use of novel polyamine transport (PAT) inhibitor compounds with pharmacological or agricultural uses and as probes for biochemical assays or for purification of selected polyamine binding targets. As drugs, these compounds are used to treat disorders of undesired cell proliferation, primarily cancer, alone or combined with other agents such as polyamine synthesis inhibitors.

The invention also relates to the synthesis and use of such novel polyamine compounds as part of combinatorial libraries. These libraries are used to discover compositions that inhibit PAT and/or that bind to a cellular polyamine transporter (PATr). Various members of these libraries or compounds discovered through use of the libraries have utility as drugs, agricultural chemicals, and as probes.

BACKGROUND OF THE INVENTION

Decades of research on the myriad of biological activities that the polyamines, putrescine, spermidine and spermine play in cellular processes have shown the profound role they play in life (Cohen, S.S., "A Guide to the Polyamines" **1998**, Oxford University Press, New York). As polycations at physiological pH, they bind tightly to and strongly modulate the biological activities of all of the anionic cellular components. Specific and strong interactions have been associated with DNA and RNA together with their associated chromatin proteins (Tabor, H. et al. 1,4-Diaminobutrane (putrescine), spermidine, and spermine. *Ann Rev. Biochem.* **1976**, *45*, 285-306; Matthews, H.R. Polyamines, chromatin structure and transcription. *BioEssays*, **1993**, *15*, 561-566). Spermine has been shown to function directly as a free radical scavenger that protects DNA from insults by reactive oxygen species (Ha, H.C. et al. *Proc. Natl. Acad. Sci. USA*, **1998**, *95*, 11140-11145). Specific interactions of multicationic polyamines with microtubules has been recently

shown (Wolff, J. Promotion of Microtubule Assembly by Oligocations: Cooperativity between Charged Groups. *Biochemistry*, **1998**, *37*, 10722-10729; Webb, H.K. et al., *J. Med. Chem.* **1999**, in press). Allosteric regulation of membrane-bound enzymes including acetylcholinesterase has been shown (Kossorotow, A. et al. Regulatory effects of polyamines on membrane-bound acetylcholinesterase. *Biochem. J.* **1974**, 144, *21-27).* Polyamines have a direct influence on many neurotransmitter receptors and ion channels (Carter, C. The Neuropharmacology of Polyamines, **1994**, Academic Press, San Diego, CA; Williams, K. Interaction of polyamines with ion channels, *Biochem. J.*, **1997**, *325*, 289-297). Specific polyamine binding sites have also been demonstrated for the NMDA receptor complex (Ransom, R.W. et al. Cooperative modulation of [^{3}H]MK-801 Binding to the *N*-Methyl-D-Aspartate Receptor-Ion Channel Complex by L-Glutamate, Glycine, and Polyamines. *J. Neurochem.* **1988**, *51*, 830-836; Williams, K. et al. Minireview: Modulation of the NMDA receptor by polyamines. *Life Sci.* **1991**, *48*, 469-498).

Many stimuli involved in both normal and neoplastic growth activate the polyamine biosynthetic pathway. A great number of multidisciplinary studies have shown that the intracellular concentrations of the polyamines is highly regulated at many steps in their biosynthesis, catabolism and transport. The fact that cells contain such complex apparatus for the tight control of the levels of these molecules shows that only a very narrow concentration range is tolerated. Ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine biosynthesis, catalyzes the production of putrescine from its precursor ornithine. This enzyme, with a very short biological half-life, is one of the most inducible mammalian enzymes known (Russell, D. et al. Amine synthesis in rapidly growing tissues: ornithine decarboxylase activity in regenerating rat liver, chick embryo, and various tumors. *Proc. Natl. Acad. Sci. USA* . **1968**, *60*, 1420-1427). Many biological stimuli involved in cellular growth have been shown to induce this enzyme and a distinct growth advantage is gained by induction of ODC (Alhonen-Hongisto, L. et al. Tumourigenicity, cell-surface glycoprotein changes and ornithine decarboxylase gene pattern in Ehrlich ascites-carcinoma cells. *Biochem. J.* **1985**, *229*, 711-715). An increase in the activity of ODC has been associated with tumor growth (Jänne, J. et al. Polyamines in rapid growth and cancer. *Biochim. Biophys. Acta* **1978**, *473*, 241-493; Scalabrino, G. et al. Polyamines in mammalian tumors. Part I. *Adv. Cancer Res.* **1981**, *35*, 151-268; Scalabrino, G. et al.

Polyamines in mammalian tumors. Part II. *Adv. Cancer Res.* **1982**, *36*, 1-102). Feedback inhibition of ODC activity is mediated by ODC-antizyme protein. Following elevation of polyamine concentrations, a polyamine-stimulated +1 frameshift of the ODC-antizyme mRNA reading frame causes elevation of this ODC-inhibiting protein (Hayashi, S. et al. Ornithine decarboxylase antizyme: a novel type of regulatory protein. *TIBS*, **1996**, *21*, 27-30; Matsufuji, S. et al. *EMBO Journal*, **1996**, *15*, 1360-1370). The ODC-antizyme protein binds to ODC with high affinity to form an inactive complex that is then tagged for degradation in an ATP-dependent fashion by the 26S proteosome (Heller, J.S. et al. *Proc. Natl. Aced. Sci. USA.* **1976**, *73*,1858-1862; Murakami, Y. et al. Ornithine decarboxylase is degraded by the 26S proteosome without ubiquitination. *Nature*, **1992**, *360*, 597-599). ODC-antizyme also represses the polyamine uptake system of cells (Suzuki, T. et al. Antizyme protects against abnormal accumulation and toxicity of polyamines in ornithine decarboxylase-overproducing cells. *Proc. Natl. Acad. Sci. USA.* **1994**, *91*, 8930-8934).

The polyamine catabolism pathway is important to prevent the toxic effects of excess polyamines on cells (Seiler, N. Functions of polyamine acetylation. *Can. J. Physiol. Pharmacol.* **1987**, *65*, 2024-2035; Seiler, N. Polyamine oxidase, properties and functions. *Progress in Brain Res.* **1995**, *106*, 333-344). This pathway is used by the cell to interconvert the various polyamines and to eliminate excess polyamines before they reach toxic levels. This pathway introduces no additional carbon precursors into the polyamine pool.

Polyamine transport into mammalian cells is energy and temperature dependent, saturable, carrier mediated and operates against a substantial concentration gradient (Seiler, N. et al. Polyamine transport in mammalian cells. *Int. J. Biochem.* **1990**, *22*, 211-218; Khan, N.A.; Quemener, V. et al. Characterization of polyamine transport pathways, in *Neuropharmacology of Polyamines* (Carter, C., ed.), **1994**, Academic, San Diego, pp. 37-60). Ample experimental proof exists that polyamine concentration homeostasis is mediated via this transport system. Changes in the requirements for polyamines in response to growth stimulation is reflected by increases in the transport activity. Stimulation of human fibroblasts to cell proliferation by serum or epidermal growth factor was followed by an 18-100 fold increase in the uptake of putrescine (DiPasquale, A. et al. Epidermal growth factor stimulates putrescine transport and ornithine decarboxylase activity in

cultures human fibroblasts. *Exp. Cell Res.* **1978**, *116*, 317-323; Pohjanpelto, P. Putrescine transport is greatly increased in human fibroblasts initiated to proliferate. *J. Cell Biol.* **1976**, *68*, 512-520). Tumors have been shown to have an increased rate of putrescine uptake (Volkow, N. et al. Labeled putrescine as a probe in brain tumors. *Science*, **1983**, *221*, 673-675; Moulinoux, J-P. et al. Biological significance of circulating polyamines in oncology. *Cell. Mol. Biol.* **1991**, *37*, 773-783). Inhibition of polyamine biosynthesis in cells in culture by α-difluoromethylornithine (DFMO), a well-studied mechanism-based inhibitor of ODC, causes a substantial depletion of intracellular putrescine and spermidine with resultant cell growth inhibition. Upon supplementing the culture media with exogenous polyamines this depletion causes transport activity to rise several-fold (Bogle, R.G. et al. Endothelial polyamine uptake: selective stimulation by L-arginine deprivation or polyamine depletion. *Am. J. Physiol.* **1994**, *266*, C776-C783; Alhonen-Hongisto, L. et al. Intracellular putrescine deprivation induces uptake of the natural polyamines and methylglyoxal bis(guanylhydrazone). *Biochem. J.* **1980**, *192*, 941-945). The cells then returned to their original rate of growth.

Several experimental lines of evidence support the conclusion that increased effectiveness of ODC inhibition can be obtained by interfering with the polyamine transport apparatus. A mutant L1210 leukemia cell line was shown to have greatly reduced polyamine transport activity following selection for resistance to methylglycoxal bis(guanylhydrazone) (MGBG), an extremely cytotoxic AdoMetDC inhibitor that is taken up by the same transport system as the polyamines. Mice inoculated with these cells had a much greater response to DFMO treatment (87% increase in median survival time; 13 of 40 mice cured) than mice inoculated with the parental cell line (22% increase in median survival time). See Persson, L. et al. Curative effect of d,l-2-difluoromethylornithine on mice bearing mutant L1210 leukemia cells deficient in polyamine uptake. *Cancer Res.* **1988**, *48*, 4807-4811. A significant source of extracellular polyamines is produced by the microbial flora in the gastrointestinal tract (Sarhan, S. et al. The gastrointestinal tract as polyamine source for tumor growth. *Anticancer Res.* **1989**, *9*, 215-224). When this source of polyamines is removed by decontamination of this flora, DFMO's previous moderate growth inhibitory effects on Lewis lung carcinoma cells or L1210 zenografts is markedly potentiated (Hessels, J. et al. Limitation of dietary polyamines and arginine and the

gastrointestinal synthesis of putrescine potentiates the cytostatic effect of a-difluoromethylornithine in L1210 bearing mice. *Int. Symp. Polyamines in Biochemical and Clinical Research*, Sorrento (Italy), **1988**, Abstr. P105). An additional source of polyamines is from dietary sources (Bardocz, S. et al. Polyamines in food; implications for growth and health. *J. Biochem Nutr.* **1993**, *4*, 66-71). By feeding a polyamine-free diet to DFMO-treated nude mice the MCF-7 human breast cancer zenografts contained greatly reduced levels of putrescine in comparison to DFMO treatment alone (Levêque, J. et al. The gastrointestinal polyamine source depletion enhances DFMO induced polyamine depletion in MCF-7 human breast cancer cells in vivo. *Anticancer Res.* **1998**, *18*, 2663-2668). In additional animal models, complete polyamine deprivation also enhanced DFMO's growth inhibitory effectiveness (Moulinoux, J.P. et al. Inhibition of growth of the U-251 human glioblastoma in nude mice by polyamine deprivation. *Anticancer Res.* **1991**, *11*, 175-180; Quemener, V. et al. Polyamine deprivation enhances antitumoral efficacy of chemotherapy. *Anticancer Res.* **1992**, *12*, 1447-1454; Chamaillard, L. et al. Polyamine deprivation prevents the development of tumour-induced immune suppression. *Br. J. Cancer* **1997**, *76*, 365-370).

The Polyamine Transporter (PATr)

The increased demand for polyamines by rapidly growing, transformed cancer cells is only partially met by an increased rate of synthesis. To exploit this increased need for polyamines, synthesis inhibitors have been sought. Additionally, lowering polyamine concentrations can result in aberrations in chromatin structure leading to cell death or inhibition of proliferation (Quemener, V. *et al.*, *Anticancer Res.* 14:443-448, 1994; Porter, C. W. *et al.*, *Cancer Res.* 53:581-586, 1993). It has become increasingly apparent that the initial disappointing results observed in the clinic with polyamine synthesis inhibitors arises from compensatory increases in transport of polyamines by a specific active transport system (Seiler, N. *et al., Int. J. Biochem.* 22:211-218, 1990; Seiler, N. *et al., J. Biochem. Cell. Biol.* 28:843-861, 1996). The promising results observed in cell culture with a suicide substrate inhibitor of ornithine decarboxylase, α-difluoromethylornithine (DFMO), or with an inhibitor of S-adenosylmethionine decarboxylase, methylglyoxal bis(guanylhydrazone) (MGBG) did not transfer to human clinical trials (Schecter, P.J. *et al.*, In *Inhibition of Polyamine Metabolism. Biological Significance and Basis for New Therapies*; McCann,

P.P. et al., eds; 1987, pp 345-364). Since the only two avenues for carbon transfer into polyamine pools are synthesis or transport, simultaneous inhibition of both of these pathways is considered by the present inventors to be a promising anti-cancer therapeutic approach.

A study confirming the validity of this chemotherapeutic approach used transplanted murine L1210 leukemia cells that were deficient in PAT. Mice transplanted with the wild-type L1210 cancer cells (with intact PAT) died after 12 days, even when treated with DFMO. In contrast, DFMO mice transplanted with PAT-deficient L1210 cells lived longer than 60 days (Ask, A. *et al., Cancer Lett.* 66:29-34, 1992). These authors also showed that treatment of mice harboring wild-type L1210 cells with a combination of (1) DFMO (2) a low polyamine diet and (3) antibiotics (which decrease polyamine production by gut flora) resulted in prolonged survival compared to treatment with DFMO alone.

Augmented PAT into cancer cells promotes cell killing. J.L. Holley *et al. (Cancer Res.* 52:4190-4195, 1992) showed up to a 225-fold increase in cytotoxicity of a chlorambucil-spermidine conjugate compared to chlorambucil alone. A series of nitroimidazole-polyamine conjugates were also effective (Holley, J.L. *et al., Biochem. Pharmacol.* 43:763-769, 1992). Others showed that mice infected with a multi-drug resistant strain of malaria were cured by treatment with a chloroquinoline-putrescine conjugate (Singh, S. *et al., J. Biol. Chem.* 272:13506-13511, 1997). Thus, the effectiveness of cytotoxic compounds could be enhanced by their conjugation with polyamines. These effects may have been due to the exploitation of the PAT system to deliver these compounds into cancer cells.

The gene for the polyamine transport protein has been cloned from *Escherichia coli* and recently from yeast (Kashiwagi, K. et al. *J. Biol. Chem.* **1990**, *265*, 20893-20897; Tomitori, H. et al. Identification of a gene for a polyamine transport protein in yeast. *J. Biol. Chem.* **1999**, *274*, 3265-3267). The genes for the mammalian transporter await identification. The transporter from *E. coli* has been crystallized and its X-ray structure has been determined (Sugiyama, S. et al. Crystal structure of PotD, the primary receptor of the polyamine transport system in Escherichia Coli. *J. Biol. Chem.* **1996**, *271*, 9519-9525). This structure represents one of only a few but growing number determined for spermidine-binding proteins. Since this structure was determined on a prokaryotic species its use in the

design of mammalian transport inhibitors was deemed to be of limited value. Despite this, several insights were obtained and used through analysis of this structure. In addition to the expected presence of carboxylate residues positioned to form salt bridges with the protonated amino groups of spermidine, numerous aromatic residues, especially tryptophan residues appeared to strengthen hydrophobic interactions with the methylene groups of the substrate. Additionally, a H_2O molecule was positioned at one end of spermidine substrate, providing stronger interactions with the ionic residues in this position.

Several researchers have studied the ability of polyamine analogs to inhibit the uptake of ^{3}H-spermidine into cells. Bergeron and coworkers studied the effect of addition of different alkyl group substitution on the terminal nitrogen atoms of spermidine or spermine analogs (Bergeron, R.J. et al. Antiproliferative properties of polyamine analogues: a structure-activity study. *J. Med. Chem.* **1994**, *37*, 3464-3476). They showed larger alkyl groups diminished the ability to prevent uptake of radiolabeled spermidine. They later concluded that increases in the number of methylenes between the nitrogen atoms decreased the ability to compete for ^{3}H spermidine uptake (Bergeron, R.J. et al. A comparison of structure-activity relationships between spermidine and spermine antineoplastics. *J. Med. Chem.* **1997**, *40*, 1475-1494). Of greater importance to the present work was their conclusion that the polyamine transport apparatus requires only three cationic centers for polyamine recognition and transport (Porter, C.W. et al. *J. Cancer Res.* **1984**, *44*, 126-128). Two groups analyzed literature examples of the polyamine analogs ability to inhibit ^{3}H spermidine uptake into L1210 cells by CoMFA and QSAR methods (Li, Y. et al. Comparative Molecular field analysis-based predictive model of structure-function relationships of polyamine transport inhibitors in L1210 cells. *Cancer Res.* **1997**, *57*, 234-239; Xia, C.Q. et al. QSAR analysis of polyamine transport inhibitors in L1210 cells. *J. Drug Target.* **1998**, *6*, 65-77).

Polyamine Transport (PAT) Assays

There is no known high-throughput assay for measuring PAT. A radiochemical assay is used for biochemical analysis of transport and has been used to study PAT in yeast and a variety of mammalian cells (Kakinuma, Y. *et al., Biochem. Biophys. Res. Comm.* 216:985-992, 1995; Seiler, N. *et al., Int. J. Biochem. Cell Biol.* 28:843-861, 1996). See, for example Huber, M. *et al. Cancer Res.* 55:934-943, 1995.

The radiometric assay uses radiolabeled polyamines such as putrescine, spermidine or spermine, but, due to the low signal, large numbers of adherent or non-adherent cells are required. Additional care is required with spermine due to its non-specific adsorption to cells and plastics. Cells are mixed with the test compounds and the radiolabeled polyamine to initiate the assay. The cells are incubated for 1-60 minutes, depending on cell type. The assay is terminated by removal of the medium and cooling the plates to 4°C. The cells are then washed with cold medium three times, dissolved in 0.1% sodium dodecyl sulfate and the radioactivity in solution is then determined by scintillation counting. This assay is difficult to scale up to a high throughput procedure due to the low signal from the radiolabel and the handling requirements inherent in procedures with radioactivity.

A great number of polyamine amide natural products have been recently been discovered in the venom of arthropods such as spiders and wasps. These acylpolyamine analogs have been shown to have specific and strong interactions with the neuromuscular junctions of insects (Moya, E. et al. Syntheses and neuropharmacological properties of arthropod polyamine amide toxins. *Neuropharmacology of Polyamines* (Carter, C., ed.), 1994, Academic, San Diego, pp. 167-184). With this capability these toxins give the insect predators the ability to paralyze or kill their prey. Most of these natural products have the common molecular features of a polyamine moiety (many with structurally diverse polyamine analogs) connected through an amide with an aromatic amino acid structural analog. Simpler synthetic analogs have been sought that attempt to maximize interactions with either crustacean neuromuscular synapses or mammalian glutamate receptors (Asami, T. et al. Acylpolyamines mimic the action of Joro spider toxin (JSTX) on crustacean muscle glutamate receptors. *Biomedical Res.* **1989**, *10*, 185-189; Raditsch, M. et al. Polyamine spider toxins and mammalian *N*-methyl-D-aspartate receptors. Structural basis for channel blocking and binding of argiotoxin$_{636}$. *Eur. J. Biochem.* **1996**, *240*, 416-426; Tsubokawa, H. et al. Effects of a spider toxin and its analogue on glutamate-activated currents in the nippocampal CA1 Neuron after ischemia. *J. Neurophys.* **1995**, *74*, 218-225).

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not constitute any admission as to the correctness of the dates or contents of these documents.

SUMMARY OF THE INVENTION

The present invention is directed to various polyamine analogues and derivatives and their use as drugs, as agricultural or as environmentally useful agents. The invention defines sites and structures within these compounds that are key to their binding (and polyamine binding) to membrane (and soluble) proteins, particularly the PATr.

The compositions of the present invention include polyamine derivatives substituted at one or more positions. Monosubstituted polyamines are preferably substituted at a terminal nitrogen, but may be alternatively or additionally substituted at internal nitrogen and/or internal carbon atoms.

A preferred embodiment is a highly specific PAT inhibitor with pharmaceutical utility as an anti-cancer chemotherapeutic. These include polyamine derivatives comprised of two linear polyamines linked to each other. The two polyamines may be identical or different and may be substituted at an internal carbon and/or nitrogen atom. Preferably, one terminal position of each polyamine is used in the linkage. The other terminal position may also be substituted.

Preferred substituents are structures that increase binding affinity or otherwise enhance the irreversibility of binding of the compound to a polyamine binding molecule, such as the PATr, an enzyme or DNA. Such additional substituents include the aziridine group and various other aliphatic, aromatic, mixed aliphatic-aromatic, or heterocyclic multi-ring structures. Reactive moieties which, like aziridine, bind irreversibly to a PATr or another polyamine binding molecule, are also within the scope of this invention. Examples of reactive groups that react with nucleophiles to form covalent bonds include chloro-, bromo- and iodoacetamides, sulfonylfluorides, esters, nitrogen mustards, *etc.* Such reactive moieties are used for affinity labeling in a diagnostic or research context, and subserve pharmacological activity as sites within a drug that inhibit PAT or polyamine synthesis. The reactive group can be a reactive photoaffinity group such as an azido or benzophenone group. Chemical agents for photoaffinity labeling are well-known in the art (Flemming, S.A., *Tetrahedron* 51:12479-12520, 1995). Photoreactive compounds for cancer treatment are also known in the art.

More specifically, a polyamine analogue or derivative of the invention includes one that binds to a polyamine-binding site of a molecule and/or inhibits polyamine transport, and has the formula

$$R_1\text{-}X\text{-}R_2$$

wherein

R_1 and R_2 are each a polyamine, or analogue or derivative thereof; and

X is a linker moiety connecting the two polyamines.

Polyamine analogues or derivatives, preferably having a reactive group at one end, may also be employed as assay or biochemical probes.

Additional substituents which may be present on the polyamine portion of analogues or derivatives (with or without a reporter group), are structures which increase binding affinity, or otherwise enhance the irreversibility of binding of the compound to a polyamine binding molecule, such as a PATr, an enzyme or DNA. Such additional substituents include the aziridine group and various other aliphatic, aromatic or heterocyclic multi-ring structures.

A reactive moiety, which, like aziridine, can bind irreversibly to a PATr or another polyamine binding molecule is also contemplated. Examples of groups which react with nucleophiles to form covalent bonds include chloro-, bromo- and iodoacetamides, sulfonylfluorides, esters, nitrogen mustards, *etc.* Such reactive moieties are used for affinity labeling in a diagnostic or research context, and subserve pharmacological activity as parts of drugs that inhibit PAT or polyamine synthesis. The reactive group can also be a reactive photoaffinity group such as an azido- and benzophenone group. Chemical reagents in photoaffinity labeling are well-known (Flemming, S.A., *Tetrahedron* 51:12479-12520, 1995). Moreover, photoreactive compounds for cancer treatment are known in the art.

The polyamine analogues and derivatives of the invention may be categorized in a variety of ways. One category of polyamine analogues and derivatives is the bispolyamines, which may be viewed as analogues or derivatives containing two linked polyamines, which may be identical or different. In preferred embodiments, the individual polyamine groups are linear and have two terminal amino groups. Examples of such polyamines include the naturally occurring polyamines: putrescine, spermidine, and

spermine. One terminal amino group in each of such polyamines may be used in the linkage between the two individual polyamines. The remaining terminal amino group may be left as an amine group or further derivatized.

Examples of polyamines for linkage into bispolyamines include N^1-dansylspermine (also termed monodansylspermine or MDS (1), N^1-dansylspermidine (also termed monodansylspermidine or MDSd, N^1-[(N^6-dansyl)-6-aminocaproyl]spermine (termed DACS, 4), N^1-[(N^6-dansyl)-6-aminocaproyl]spermidine (DACSd), N^1-[(N^6-5-(4-chlorobenzamidomethyl)thiophene-2-sulfonyl)-6-aminocaproyl]spermine 5 or N^1-[(N^6-(2-dibenzofuransulfonyl)-6-aminocaproyl]spermine 6.

Additional polyamines for linkage into bispolyamines include N^1-acyl aminoacid-spermine conjugates. These include natural and non-natural amino acid amides of spermine which are by themselves very effective polyamine transport inhibitors. Examples of such polyamines include L-Lys-spermine (compound 1202), L-Val-spermine (compound 1157) and L-Orn-spermine (compound 1224).

More polyamines for linkage into bispolyamines are acyl polyamines, such as N^1-monosubstituted. Monosubstituted polyamines can be further classified into categories such as amides, sulfonamides, N^1-monosubstituted amines and other. Among the amides, further classification into those without linkers, those with linkers, amino alkyls, and amino acid head groups is possible. The amino acid head groups can be further categorized as those that are protected, natural α- amino acids, non-natural α-amino acids, and amino acid derivatives.

Once a polyamine analogue which inhibits polyamine transport at a desirable level has been identified, it can readily be further optimized by structural and functional comparisons with other polyamine analogues in the same or different categories to improve its utility. Examples of such improvements include, but are not limited to, increased inhibitory activity, enhanced metabolic stability, enhanced specificity, ease of handling and administration, binding affinity, non-incorporation into cellular polyamine pools, and decreases in side effects.

The present invention is also directed to a pharmaceutical composition useful for treating a disease or condition in which the inhibition of polyamine transport is desirable, comprising a composition as described above and a pharmaceutically acceptable excipient.

The pharmaceutical composition may further include an inhibitor of polyamine synthesis; preferably DFMO. Other combinations include the above pharmaceutical composition and one or more additional agents known to be useful for treating said disease or condition

This invention also provides a method for treating a disease or a condition in a subject associated with undesired cell proliferation and/or which is treatable by inhibition of polyamine transport, comprising administering to said subject an effective amount of a pharmaceutical composition as described above. The undesired cell proliferation may be associated with proliferation of cells of the immune system, cell of the vascular neontima, tumor cells or with undesired angiogenesis. Preferred diseases to be treated as above include cancer or post-angioplasty injury.

Thus the analogues and derivatives of the invention, alone or in combination with other agents, may be used for the treatment of cancer and other diseases of unwanted cellular proliferation, including angiogenesis and post-injury cell growth. Preferably, such treatments act by inhibiting PAT, deoxyhypusyl synthase, or cell growth or by the induction of apoptosis. As such, they may act by cytostatic and/or cytotoxic mechanisms. The analogues and derivatives of the invention, individually or in combinations with or without other agents, may also be used to treat hypertension, osteoporosis, Alzheimer's disease, ischemia, autoimmune diseases, psychosis, depression, strokes, cardiovascular disease, infection with microorganisms or parasites, plant pathogens including fungi. Cellular processes susceptible to inhibition by the analogues and derivatives of the invention, alone or in combination with other agents, include those involving nucleic acids (DNA or RNA), such as replication, transcription or translation. The analogues and derivatives of the invention may also be efficacious as anti-diarrheal, anti-peristaltic, anti-spasmodic, anti-viral, anti-psoratic and insecticidal agents.

The invention is also directed in part to rapid and efficient testing of many such analogues and derivatives for their transport into cells. By creating a database of structure-activity-relationships (SARs) of such analogues and derivatives, the invention identifies elements that are key for polyamine binding to membrane proteins such as PATr or soluble proteins. With such information, the invention permits predictions as to the transportability and activity of novel polyamine analogues and derivatives.

The polyamine analogues and derivatives of the invention may also be employed as assay or biochemical probes. A preferred assay method employs a polyamine analogue or

derivative with a moiety that serves as a detectable label (a "reporter"), preferably a fluorophore, most preferably the dansyl group, or another substituent that can be detected through a variety of means, including by ELISA. A preferred assay method employs an analogue or derivative immobilized to a solid support.

The present invention is also directed to a series of polyamine analogues useful in diagnostic compositions. Methods for the synthesis of such compounds are also described.

Details concerning SARs databases, the use of polyamine analogues as assay probes, and diagnostic compositions are set forth in PCT/US98/14896.

The invention further identifies elements that are key for polyamine binding to membrane proteins such as the PATr (PATr), and to soluble proteins.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the structure and activity relationships (SAR) between spermidine, MDS and DACS. K_i values are the inhibitory constants obtained in a PAT inhibition assay.

Figure 2 is a tabular representation of a large number of chemical structures **3-98** that were tested for their effects on cell growth. R, an index of growth inhibitory activity, is the ratio of the growth of cells in the presence of the test compound to the growth in the presence of the compound plus DFMO. The K_i, (inhibition constant) reflects a compound's inhibition of PAT in cell culture. These biological effects provide a basis for SAR analysis.

Figure 3A shows a representative synthetic route for the preparation of bispolyamines of the invention. In this synthetic scheme, a bispolyamine containing two N^1-^{t}Boc-spermine polyamines is produced by linking the spermine derivatives with 4-nitrophenyl ester. The crude product from this reaction, after removal of the methanol (MeOH) and dimethyl formamide (DMF) solvents by evaporation and/or high vacuum, can be dissolved in either water or 50% MeOH/water for purification by column chromatography such as with a cation exchange column. Elution was with a gradient ranging from 0 to 1 or 2N NH_4OH.

Figure 3B shows the same representative synthetic route as Figure 3A but with a further step of removing the N^1-^{t}Boc protecting group with 3M HCl.

Figure 4 shows representative synthetic routes for the preparation of p-nitrophenyl activated esters by conversion from the corresponding acid chlorides.

Figure 5 shows a representative reaction for the protection of a terminal amine group in spermine by treatment with di-tert-butyldicarbonate.

Figure 6 shows preferred polyamine analogs of the invention that may be linked to form a bispolyamine.

Figure 7 shows the general structure of bis-amide dimers of spermine linked by an aliphatic or aromatic di-acid chain.

Figure 8 shows preferred linked bis-amide dimers of spermine.

Figures 9a to 9j contain tables classifying a large number of N^1-monosubstituted polyamines which may be used to form bispolyamines of the invention.

Figure 10 shows four classes (111-114) of conformationally restricted polyamine analogues, and at the bottom, a stereochemically defined, internally cyclic polyamine analogues (116) that may be used in the preparation of the bispolyamines of the invention.

Figure 11 shows compound 1202 L-Lys-spermine and variations of that compound, which may be used in preparing bispolyamines of the invention.

Figure 12 lists amino acid-polyamine conjugates where the amino acid moiety may vary in chirality. These amino acids may also be used to form bispolyamines of the invention.

Figures 13 and 14 show the synthesis of biotin modified polyamines N^1-[(N^6-(biotinyl)-6-aminocaproyl)]spermine and N^1-(biotinyl)spermine, which may be used in preparing the bispolyamines of the invention.

Figure 15, panels A and B, is a schematic illustration showing the possible sites for modifying a polyamine to create an "immobilization handle" and a "reporter handle" combination. These modified polyamines may be used in the present invention to produce bis-polyamines.

Figure 16 is a table of preferred deprotected bispolyamines of the invention.

Figure 17 is a table of preferred protected bispolyamines of the invention.

Figure 18 shows some activated esters suitable for preparation of bispolyamines.

DETAILED DESCRIPTION

The present inventors have designed novel compounds for therapeutic uses and have devised tests using such compounds as probes for measuring PAT and polyamine binding in an efficient, high throughput assay. Using the novel methods, they have screened for and discovered compounds with high affinity for the PATr that inhibit uptake, both competitively and non-competitively. Such compounds are useful as drugs in a number of diseases, particularly cancer. They can also be used as a component of novel drug combinations with, for example, a polyamine synthesis inhibitor such as DFMO (which inhibits ornithine decarboxylase) or with other agents. The compounds of the present invention are also useful in other diseases or conditions in which polyamines play a role as described above, and have agricultural and environmental uses.

The inventors found that formation of a bispolyamine from individual polyamines give it advantageous properties as an inhibitor of PAT or as a probe in an assay of PAT and for drug screening. Such chemical modification does not destroy the effective binding and, in fact, may enhance the affinity of the derivatized polyamine for the PATr. Hence, these compounds are useful for discovery of inhibitors of polyamine uptake.

Definitions

As used herein, the term "polyamine" includes putrescine, spermine or spermidine, as well as longer linear polyamines, branched polyamines, and the like, which may have between 2 and about 10 nitrogens. Also included in this definition are polyamine derivatives or analogues comprising a basic polyamine chain with any of a number of functional groups bound to a C atom or a terminal or internal N atom. A polyamine derivative may include a terminal linker or spacer group between the polyamine core and a derivatizing function.

A "head group" is defined as a moiety bonded either directly to the polyamine or attached to a linker that is bonded to the polyamine. It is preferably an aromatic or heterocyclic group, although aliphatic groups or aroalkyl groups are included. Thus, a head group may be a fluorescent moiety, which also serves as a "reporter."

An "inhibitor" moiety or group is a chemical group derivatizing a polyamine that (1) causes the derivative to bind to the PATr with higher affinity than does a native polyamine and/or (2) by other means blocks the uptake of a polyamine (or a probe of this invention) into a cell or a subcellular PATr preparation. The inventors disclose herein

compounds that efficiently inhibit PAT in MDA-MB-231 human breast carcinoma cell and other cells. A number of different types of such inhibitors have been synthesized; various of the synthetic schemes are disclosed herein.

A "reporter moiety" is a chemical moiety forming part of a probe which renders the probe detectable (either directly or, for example, through enzymatic enhancement) and hence permits the determination of the activity of the PATr to which the probe binds. A reporter is detectable either because it itself emits a detectable signal, or by virtue of its affinity for a reporter-specific partner which is detectable or becomes so by binding to, or otherwise reacting with, the reporter. In a preferred embodiment the polyamine analogue is immobilized to a solid support which enables removal of the analogue and any interacting/binding molecules from a complex mixture.

The various inhibitor compounds disclosed herein are identified by various numerical designations, including a counting scheme (using values from 1 to 166 and above) and an identifier number scheme (using four digit compound numbers alone or in combination with an "ORI" or "Ori" identifier). Irrespective of what identifying scheme is used, the identifier merely represents the actual molecular structure of the compound involved and imposes no limitation on said compound.

Overview of Structure-Activity Relationships (SARs)

The PAT inhibitors were developed by modification of the natural substrate of the transporter, spermidine. The present inventors discovered that introduction of a 3-amidopropyl group to the diaminobutyl part of spermidine produced a significantly better transport inhibitor as shown in Figure 1. The optimal amido or sulfonamide substituent was found to be a medium sized aromatic group, leading to the invention of N^1-dansylspermine (MDS) as both a transport inhibitor and a transport assay reporter molecule. MDS has increased binding affinity to cells compared to spermidine and N^1-acetylspermine. Significantly enhanced inhibition of cell growth and PAT resulted from the introduction of a 6-carbon atom linker between the aromatic "head" group of MDS and the polyamine core. This new molecule, N^1-[(N^6-dansyl)-6-aminocaproyl]spermine (or DACS) 4, is one of the most potent PAT inhibitors known. In its interaction with biological systems, DACS shows many of the desired properties set forth above. The present inventors have studied DACS and other related analogues extensively.

The SARs around DACS 4 as a lead compound have been explored extensively as shown in Figure 2 (in particular, compounds 73-98). As discussed above, changes were made in each of several regions of DACS, and effects on transporter binding were measured. The impact of changing the aromatic "head" group was explored by synthesizing a number of different activated 4-nitrophenyl esters with different aromatic and non-aromatic N-sulfonamides at the distal amino end. Another series of "headless" analogues were synthesized to explore the importance of the hydrophobic aromatic grouping. In sum, the present inventors have designed and synthesized a large number of compounds that efficiently inhibit PAT. As described herein, all mono, di and multi-substituted polyamines with the various substituents are intended for use as drugs.

N^1-substituted polyamine analogues may be prepared as described in related applications U.S. 09/341,400 and 09/396,523, which often presents representative reactions with spermine as a non-limiting example of a polyamine core for use in the present invention. The preferred mono-protected polyamine intermediates for use in preparing bispolyamines were the amino terminal tBoc derivatives produced according to Blagbrough *et al.*, (*Tetrahedron Lett.* 35:2057-2060, 1994), using di-*tert*-butyldicarbonate in tetrahydrofuran.

Lead polyamine analogue compounds may be further modified to produce analogues for the production of bispolyamines. For example, following structural explorations around the amide, sulfonamide or urea substituent, it was determined that introduction of a six carbon, straight chain aliphatic linker between the polyamine core and the head group led to a 10-fold increase in binding to the PATr (see Figure 1). Given the high affinity this compound, DACS 4, to its biological target, it was selected as a lead compound for further modification. Methods for the further derivatization of this lead compound is described in related applications U.S. 09/341,400 and 09/396,523.

A fruitful general approach to realize selectivity of binding to a target (*e.g.*, protein) of interest has been to synthesize conformationally or stereochemically defined analogues of a binding molecule. By significantly reducing the number of possible rotomers or conformations a molecule can adopt, one can attain increased binding to the desired site. Since the molecule no longer has to search the entire "conformational space," its energy of interaction with the target increases many times.

Others have tried to solve the selectivity problem with polyamine analogues by synthesizing conformationally restricted analogues. Ganem replaced the butyl portion of spermine with 2-butene and 2-butyne diamino derivatives (Ganem, B., *J. Org. Chem.* 1987, *52*, 5044-5046). Rajeev, K.G. *et al.*, *J. Org. Chem.* 1997, 62, 5169-5173, incorporated a stereochemically defined, conformationally restrained pyrrolidine ring into the spermine backbone (Fig. 10; 115, x=1) Brand, G. *et al.*, *Tetrahedron Lett.* 1994, 35, 8609-8612, synthesized cyclopolyamine analogues of spermidine and spermine. See, for example Figure 10 (113, x= 3, 4, and 5). The present inventors extended this work by producing the other analogues shown in Figure 10. These analogues are synthesized using variations of known methods. The analogues where x = 1 are produced by reacting spermine or N,N'-bis(3-aminopropyl)-1,3-propanediamine with formaldehyde as described by Ganem, B., *Acc. Chem. Res.*, 1982, *15*, 290). The primary amines are protected as N-tBoc derivatives for the analogues 111 and 113. Acid deprotection then gives the desired products. The derivative 112, where x = 1, was also synthesized Ganem.

Analogues 111 and 113 (Figure 10), where x = 2 to 4, were produced by reductive alkylation. N^1, N^{14}-Bis(tBoc)spermine was reacted with the dialdehyde, $OHC(CH_2)_{x-2}CHO$ and $NaBH_4$ in EtOH. Compounds 112 and 114 were made by the same procedure on a suitable N^1,N^4-bisprotected spermine derivative.

Stereochemically defined, internally cyclic structures (Figure 10, 115) are synthesized using an intermediate aldehyde produced from the corresponding alcohol. This protected alcohol can be oxidized to the aldehyde using Swern conditions. Aldehyde extension by the Wittig reaction with formylmethylene triphenylphosphorane, followed by reduction (overreduced alcohol can be reoxidized to the aldehyde using pyridinium chlorochromate) and reductive amination/cyclization completed the sequence to make the analogues where x = 2. By Wittig reaction with 3-bromopropyl triphenylphosphonium bromide, deprotection and intramolecular alkylative cyclization, the analogue where x = 3 can be produced. Either stereoisomer can be produced by starting with L- or D-ornithine. Polyamines containing a guanidinium group are synthesized according to Iwanowicz, E.J. *et al.*, *Synthetic Comm. 23* 1443-1445, 1993.

The natural polyamines, including putrescine, spermidine and spermine, are incorporated into the compositions of this invention by coupling them to various "head"

and "linker" groups. Other naturally occurring polyamines that can be employed similarly include: N^1-acetylspermine, N^1-acetylspermidine, N^8-acetylspermidine, N^1-guanidinospermine, cadaverine, aminopropylcadaverine, homospermidine, caldine (norspermidine), 7-hydroxyspermidine, thermine (norspermine), thermospermine, canavalmine, aminopropylhomospermidine, N, N'-bis(3-aminopropyl)cadaverine, aminopentylnorspermidine, N^4-aminopropylnorspermidine, N^4-aminopropylspermidine, caldopentamine, homocaldopentamine, N^4-bis(aminopropyl)norspermidine, thermopentamine, N^4-bis(aminopropyl)spermidine, caldohexamine, homothermohexamine and homocaldohexamine.

The metabolic stability *in vivo* of monosubstituted polyamine analogues is increased by modifying these compounds to resist enzymatic degradation. For example, substitution of the terminal primary amine group with an alkyl group would achieve this by preventing oxidative metabolism. This invention also includes compounds with alkylated secondary amino groups. N-alkylation of the amide nitrogens slows down proteolytic degradation.

An additional method to prevent metabolic degradation of amide bonds is to produce the thioamide derivative. Figure 11a shows these changes implemented into compound 1202 L-Lys-spermine conjugates before its use in the bispolyamines of the invention. Combinations of these changes are also encompassed as part of the present invention.

The foregoing changes can be achieved by a number of synthetic routes. Substitution of carbon atoms α to secondary nitrogens and acylation of nitrogens can also slow degradation by polyamine oxidase. Such chemical modifications may minimize potential pharmacological side effects of these compounds.

Alternatively, methyl groups can be introduced α to the terminal amino groups of spermine (Lakanen, J. R. *et al.*, *J. Med. Chem.* 35:724-734, 1992). The 1,12-dimethylspermine analogue 121 was very resistant to normal metabolic degradation. This compound is easily coupled as part of a bispolyamine.

Polyamine analogues of 4 with acetyl (47), N-ethyl (35) and α-dimethyl (66) substitution have been synthesized and shown to have K_i's (for the MDA-MB-231 cell PATr) of 2100, 41, 18 nM, respectively.

Detectably labeled polyamine derivatives can be synthesized using radiolabeled ^{14}C-spermine or other radiolabeled polyamine as starting material.

Various polyamine analogues alkylated at internal carbons can also be synthesized. 5-carboxyspermine, tetra *t*Boc-5-carboxyspermine and its acid chloride are synthesized according Huber, H. *et al., J. Biol. Chem.* 271:27556-27563, 1994. The resulting acid chloride can then be reacted with various nucleophilic reagents to produce carboxy-substituted polyamine analogues following removal of the tBoc group. These analogues can then be coupled to the reagents that donate the linker and/or head group. Alternatively, the carboxy intermediate can be reduced to an intermediate that is used to synthesize numerous analogues. Such analogues are of interest in the present invention as alkylating agents (*e.g.*, internal aziridine spermine derivatives) or as enzyme-activated irreversible inhibitors of enzymes involved in polyamine biosynthesis, utilization and degradation (*e.g.*, spermine synthase, deoxyhypusine synthase, polyamine oxidase). Any enzyme that acts on the substituted carbon atom will generate a highly reactive intermediate that can alkylate the enzyme's active site residues.

Many polyamine derivatives are available commercially, and these can easily be derivatized further to make the polyamine analogues of the present invention.

Preferred bispolyamines

Preferred bispolyamine compounds include those produced by linking polyamine analogs as presented in Figures 2 and 9a to 9j as well as derivatives thereof with pharmaceutical utility as an anti-cancer, anti-viral, anti-microbial, or anti-fungal chemotherapeutic. Particularly preferred compounds include those presented in Figures 16 and 17 as well as derivatives thereof.

The further derivatization or optimization of bispolyamine compounds having a desirable activity may be achieved by structural and functional comparisons with other bispolyamine analogues and derivatives of the invention to incorporate particular structural elements of other analogues into the compound being optimized. The structural elements will be selected based on the expectation of improving functionalities such as, but not limited to, inhibitory activity, metabolic stability, specificity, handling and administration,

binding affinity, non-incorporation into cellular polyamine pools, and decreases in side effects.

The resultant compounds modified by the introduction of such structural elements may be of any structure, including those within the limits of the bispolyamine analogues and derivative structures defined herein. Stated differently, the resultant compounds may have one or more additional atoms or functional groups and/or removal of one or more atoms or functional groups after optimization, resulting in a compound either within or beyond the limits of the bispolyamine analogues and derivative structures defined herein.

Multiple iterations of optimizing compounds with preferred activity may be conducted to further improve the bispolyamine analogue.

The design of some bispolyamine analogues and derivatives of the invention was driven by several requirements of any compound that would act in concert with an ODC inhibitor in a combination therapy to deplete cellular polyamines through both the biosynthetic and transport pathways. Such compounds need to be good inhibitors extracellular uptake of polyamines (putrescine, spermidine, and spermine) while not being themselves substrates for the transporter or for maintenance of cellular polyamine levels. If such were substrates of the transporter and could function as the natural polyamines (or be metabolized to polyamines), the compounds would defeat their purpose of depleting cellular polyamine levels.

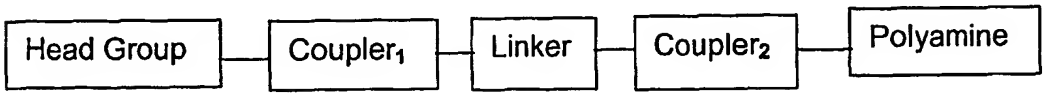
In addition to the use of amino acid groups, the bispolyamine analogues and derivatives of the invention may comprise a polyamine with a head group linked to a polyamine where coupler such as -C(=O)NH-, -S(=O)$_2$NH-, -NHC(=O)-, -HNS(=O)$_2$-, -HNC(=O)NH-, -HNC(=S)NH-, O-C(=O)NH-, -O-, -S-, -CH_2- or -NH- is used to combine the "head" group and the linker moiety.

Head Groups

1. General Description

As stated above, bispolyamines of the invention are composed of polyamine derivatives that are linked together via terminal amino groups. One example of polyamine derivatives that may be made part of a bispolyamine are polyamine lead compounds derivatized with a head group.

The general construction of the lead compounds shown below indicates the connections between the head group, linker and polyamine:



where coupler$_1$ is -C(=O)NH-, -S(=O)$_2$NH-, -NHC(=O)-, -HNS(=O)$_2$-, -HNC(=O)NH-, -HNC(=S)NH-, O-C(=O)NH-, -O-, -S-, -CH$_2$- or -NH-; and

coupler$_2$ is -C(=O)NH-, -S(=O)$_2$NH-, -HNC(=O)NH-, -HNC(=S)NH- or -NH-

A number of coupling chemistries can be used to combine the "head" group and the linker moiety. Types of "head" groups are disclosed below as are additional groups that can be substituted onto these head groups.

The coupling between the polyamine and linker will be described below before description of the linkers. What follows is the definition of the head groups.

The structural diversity of preferred head groups is very large, and most organic groups that can be covalently attached to an amine are potential candidates. The following table provides guidance regarding the intended head groups but is by no means is intended to be limiting. Additional examples of head groups suitable for use in the polyamine analogues of the invention include those in column "R2" of Table 1 in Dhainaut et al. (1996) "New purines and purine analogs as modulators of multidrug resistance." J. Med. Chem. 39:4099-4108, which is incorporated herein in its entirety as if fully set forth. Mono and multi-substitutions on the ring structures of the head groups are also intended.

LIST OF HEAD GROUP SUBSTITUENTS

halogen	cyclohexyl	ethoxyl	propyl ester
methyl	cycloheptyl	propoxyl	isopropyl ester
ethyl	cyclooctyl	thio	cyano
propyl	cyclononyl	methylthio	isocyanato
isopropyl	cyclodecyl	ethylthio	trifluoromethyl
butyl	hexyl	propylthio	trichloromethyl
isobutyl	2-hexyl	butylthio	tribromomethyl
tert-butyl	3-hexyl	isopropylthio	azido
pentyl	allyl	nitro	Acetoxy
2-pentyl	vinyl	amino	Carboxamide
3-pentyl	acetylenic	acetamide	N-methylcarboxamide
neopentyl	propargylic	formamide	N,N-dimethylcarboxamide
cyclopentyl	homopropargylic	carboxylic	N-ethylcarboxamide
cyclopropyl	hydroxyl	methyl ester	N,N-diethylcarboxamide
cyclobutyl	methoxyl	ethyl ester	

2. Aromatic Groups

Aromatic groups include phenyl naphthyl, 1-, 2-, or 3-biphenyl, indenyl, acenaphthylenyl, anthracenyl, phenanthrenyl, phenalenyl, triphenylenyl pyrenyl, diphenylmethylenyl, *etc.*

3. Heterocyclic Groups

Heterocyclic groups include pyrrolidinyl, piperidinyl, piperazinyl, morpholinyl, biphenyl, furanyl, pyrrolyl, 1,2-diazolyl, imidazolyl, 1H,1,2,3-triazolyl, 1H-1,2,3,4-tetrazolyl, thiazolyl, oxazolyl, 1,3,4-thiadiazolyl, pyridinyl, pyrimidyl, 1,2-diazinyl, 1,4-diazinyl, 1,3,5-trizinyl, dibenzofuranyl, acridinyl, 2,1,3-benzothiadiazole, isoquinolinyl, quinolinyl, benzufuranyl, isobenzofuranyl, 1,3-benzodiazinyl, phenazinyl, phenoxazinyl, phenothiazinyl, pyran, chromenyl, xanthenyl, indolizinyl, isoindolyl, indolyl, purinyl, phthalazinyl, naphthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, ptericinyl, carbazolyl, β-carbolinyl, phenanthridinyl, acridinyl, perimidinyl, phenanthrolinyl, isothiazoly, furazanyl, indolinyl, isoindolinyl, quinuclidinyl, and biotinyl.

4. Aliphatic Groups

This class includes straight-chain, branched and cyclic hydrocarbons attached to the linker. The group includes C_{2-10} alkanes; C_{3-10} alkenes containing 1 to 3 unsaturations; C_{3-10} alkynes containing 1 to 3 unsaturations; branched C_{3-10} alkanes, alkenes and alkynes; polycyclic aliphatic hydrocarbons and steroid-like ring systems that include C_{3-8} cycloalkyl, adamantyl, camphoryl, cholesteryl, *etc.*

5. Miscellaneous-

a. DNA intercalators:

Coupling an intercalator to the polyamine will yield an agent with much higher affinity for nucleic acid targets. Examples of intercalating agents amenable to this use are acridine, 9-aminoacridine, proflavine, actinomycin D, daunorubicin, doxorubicin, nogalamycin, menogaril, ellipticine, BD-40, amsacrine, acodazole, 2-pheylquinoline carboxamide, crisnatol, nitracrine, pyrazoloacridine, mitonoafide, ametantrone, mitoxantrone, oxanthrazole, bisantrene, echinomycin. For a review of DNA intercalating agents see Baguley, B.C., *Anti-Cancer Drug Design* 1991, 6, 1-35.

b. Biochemical conjugates

Drug selectivity is achieved by targeting specific cells or enzymes/receptors on cells. The following biochemicals are candidates for coupling to polyamines for producing a selective pharmaceutical agent: steroids, prostaglandins, phospholipids; enzyme cofactors including nucleotide containing molecules such as NADH, AcetylCoA, AdoMet, flavin, tryptophantryptophyl quinone (TTQ), *etc.*

An additional series of head groups comprises polyamines conjugated to polyethylene glycol (PEG) or O-methylated PEG (abbreviated MeOPEG) polymers of various sizes.

6. Multiple Ring Head Groups

Head groups can vary from simple alkyl substitutions to multi-ring and multi-single-ring substitutions. Some of the structural variations are schematically represented in Figure 15 of U.S. Patent Application 09/341,400.

Linker Group

1. General Description

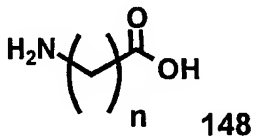
The linker portion of polyamine analogues for use in bispolyamine compounds can be represented by a general structure with an amino group at one end and an acid group on the other. One group of linkers contains diamino groups that are bonded via a urea linkage to the polyamine and via an amide, urea or sulfonamide linkage to the head group. The head group can also be bonded through other couplings such as ether, thioether and C-C bonds. The schematic structure shown above (in the section labeled "Head Groups, 1. General Description) shows the function of the linker moiety connecting the head group to the polyamine and possessing a desired length and combination of steric, conformational and hydrophobic properties. Also shown are the possible combination of coupling methods. Each coupling method can be used in combination with any of the three methods in Figure 3 of U.S. Patent application 09/341,400 at the other position to result in a wide array of desired properties.

The linker group can have a range of properties that are reflected by the number of variations discussed below. Changes in the linker structure will be affect the properties of the whole polyamine analogue such as hydrophobicity, hydrophilicity, distance between

head and polyamine portions, steric arrangement of head and polyamine portions, conformational properties, solubility and electronic properties.

2. Aliphatic Straight Chain Linkers

A series of linkers was been synthesized to test the effect of different distances between head group and polyamine. This series is most simply represented by the straight-chain aliphatic linkers having various carbon chain lengths shown below as compound 148).



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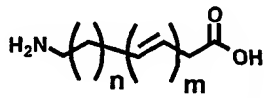
n = 1 to 12

The present inventors discovered that linker length had dramatic effects on the PAT inhibitory activity and the cell growth inhibitory activity. A low K_i is optimal for C_6 linkers in the presence of an aromatic head group. However, in the absence of a head group, differences in growth or transport inhibitory activities have not been dramatic. Thus, "headless" compounds have K_is in the order of about 25 nM but have more attenuated inhibitory effects cell growth (breast cancer cell line) most likely due to their ability to actually be transported. A prostate cancer cell line is more powerfully inhibited by these "headless" inhibitors. The C3-headless compound had dramatic effects on cell growth.

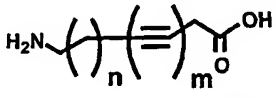
The synthetic route to this series of compounds, starting with various polyamines and head groups, is represented by the DACS 4 synthetic scheme depicted in Figure 9 of U.S. Patent Application 09/341,400). The amino group is protected by the N-^{t}Boc group, and the carboxylic acid is then activated by forming the p-nitrophenyl ester. After acid deprotection of the N-^{t}Boc group, the amino group can be reacted with an acid or sulfonamide chloride of the desired head group. After purification, direct reaction with the polyamine of choice in methanol gives the desired product. This can be purified by either (1) reverse-phase silica gel chromatography using 2:9 MeOH/0.5 N HCl or (2) cation-exchange chromatography over BioRex 70 resin (NH_4 form) using a linear gradient of from 0 to 2N NH_4OH.

3. Unsaturated straight-chain aliphatic linkers

Varying degrees of unsaturation (alkene and alkyne) together with the geometric isomers of the alkene derivatives can be introduced into the linker moiety as depicted below (149 and 150). These variations allow introduction of conformational restraint into the final product.



149



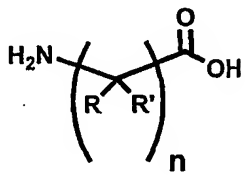
150

E and *Z* isomers

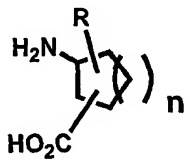
where n=0 to 7 and m=1 to 4

4. Carbon-substituted and cyclic aliphatic linkers

Branched chain and cyclic saturated aliphatic linker groups impose conformational restraint on the desired polyamine analogue. Compounds 151 and 152 below illustrates this class of structure.



151



152

where n=1-10; R and R' vary independently and can be H or $CH_3(CH_2)_m$, and where m=1 to 10.

5. Chiral carbon-substituted amino acid linkers

Great structural diversity can be incorporated quickly into the polyamine analogues by using any of the large number of chiral amino acids that are available commercially. Many of the chiral amino acid intermediates are also available commercially, including some N-^{t}Boc protected amino acids and some N-^{t}Boc protected amino acid p-nitrophenyl esters. Figure 12 (153) illustrates a variety of derivatives that have been produced by this method. These amino acid-polyamine conjugates contain variable chirality in the amino acid moiety. The amino acids can also be used as "linkers" to other N-substituted "head groups".

An additional thousand α-amino acid analogues known in the art can be used to form polyamine adducts. These are very easily incorporated into the present invention

through synthetic sequences described in Figures 8 and 9 of U.S. Patent Application 09/341,400. Several key examples are; t-butylglycine, ornithine, α-aminoisobutyric acid, 2-aminobutyric acid, α–aminosuberic acid, 4-chlorophenylalanine, citrulline, β-cyclohexylalanine, 3, 4-dehydroproline, 3, 5-diiodotyrosine, homocitrulline, homoserine, hydroxyproline, β–hydroxvaline, 4-nitrophenylalanine, norleucine, norvaline, phenylglycine, pyroglutamine, β-(2-thienyl)alanine, *etc.* Several important β-amino acids are easily incorporated into the present invention through the chemistry discussed above. A key example is β-alanine, *etc.*

Both stereoisomers of the natural L-amino acids (L= S) or D–amino acids (D=R) can be used in this invention. Because each isomer can be used individually, the structural diversity of the analogues is markedly enhanced.

6. "Headless" linkers

The desired biological properties do not always depend upon the presence of a head group. Hence, a large series of so-called "headless" derivatives, containing a polyamine and linker without a head group were synthesized and tested. These derivatives are made by reacting the active ester (p-nitrophenyl or N-hydroxylsuccinimide) of the N-^{t}Boc amino acid with the polyamine of interest. The resulting N-^{t}Boc protected derivatives are then purified by cation-exchange chromatography over BioRex 70 (NH_4 form) resin using a linear gradient from 0 to 2N NH_4OH. The ^{t}Boc group can then be cleaved by acid treatment. Both the tBoc and acid deprotected derivatives can be tested for biological activity. The full series of amino acids discussed above, together with other derivatives have been synthesized.

Reactive, Irreversible Polyamine Transport Inhibitors

A. Alkylating Reagents-

1. Aziridines

Polyamines substituted with fluorophores and other bulky end group were found to have the intrinsic property of high avidity binding to the PATr. This suggested that, in addition to utility as a diagnostic or research tool, they are useful as therapeutic agents for treating diseases or conditions wherein it is desirable to inhibit PAT. Their intrinsic affinity for other polyamine targets such as DNA broadens even further the scope of their

therapeutic utility. Correspondingly, bispolyamines containing such modified polyamines are expected to display the same activities.

In a preferred embodiment the polyamine core is substituted with the aziridinyl group. Aziridinyl-substituted polyamines react with nucleophilic groups in target binding complexes (receptors, transporters, enzymes and nucleic acids). In addition they can be exploited to bind other reactive moieties to polyamines. These mono- and di-substituted polyamine analogues are useful as drugs because of their inhibition of (a) the PATr, (b) polyamine synthesis and (c) reactions that use nucleic acids as substrates.

In another embodiment, a reactive group other than aziridine is introduced into a polyamine already substituted with a head group and a linker. This reactive group allows the labeled polyamine to bind covalently to an appropriate nucleophilic site on a polyamine-binding target molecule such as the PATr. Compounds of this type are used to covalently label receptors, enzymes or nucleic acids; thus, the modified polyamine serves as an affinity label that is useful in diagnostic assays and as a tool to isolate a polyamine binding target. Again, such compounds used as drugs will treat diseases or conditions which are ameliorated by blocking PAT or DNA-polyamine interactions. By virtue of the relative irreversibility of their binding, such compounds can be used at lower doses or at decreased frequency compared to compounds known in the art.

Disubstituted polyamines are synthesized by using the appropriate amine protecting groups on the polyamines. Reagents for the stepwise fuctionalization of spermine are known (Bergeron, R.J. *et al., J. Org. Chem. 53*: 3108-3111 (1988); Byk, G. *et al., Tetrahedron Lett. 38*: 3219-3222 (1997)). Bergeron *et al. (supra)* described the use of four independent amine-protecting groups: benzyl, t-butoxycarbonyl, trifluoroacetyl, and 2,2,2-trichloro-t-butoxycarbonyl. Conditions that allow the selective removal of each protecting group were also described. These reaction conditions allow independent and selective derivatization of each nitrogen of spermine. Thus this invention includes derivatization of monofunctionalized spermine with a linker/head group on any one of the four nitrogens and the synthesis of polyamine analogues with more than one functionalized nitrogen.

Methods to introduce an aziridine group into spermine (Li *et al, J. Med. Chem., 39*:339-341 (1996) and into derivatives of spermidine (Yuan *et al, Proc. Am. Assoc. Cancer Res., 34*: 380 (1993) are available.

2. Other Reactive Groups

Other useful moieties that can be added instead of the aziridine group and that react with nucleophiles to form covalent bonds include chloro-, bromo- and iodoacetamides, sulfonylfluorides, esters, nitrogen mustards, *etc.*

The chemically reactive 2-haloacetamide group can easily be introduced into any of the polyamine analogues by reaction with the appropriate 2-haloacetic acid halide. Other chemically reactive groups are described below.

B. Photochemically Activated Reagents

The use of photochemically activated functionalities on biologically active molecules is a well known (Fleming, S.A., *Tetrahedron 51*:12479-12520, 1995). In the polyamine field, Felschow *et al.* attached an azidobenzoic acid moiety to spermine and examined the interaction of the resulting adduct with cell surface proteins (Felschow, DM *et al. Biochem. J. 328*, 889-895, 1997; Felschow, DM *et al., J. Biol. Chem. 270:*28705-28711, 1995). Since their photoprobe had an apparent K_i of 1 μM versus spermidine for the PATr, the photolabeled proteins described were a mixture of polyamine binding proteins. One of the most potent PAT inhibitors of the present invention, DACS, has a Ki of <10 nM, which indicates an affinity 100 times higher than the compound reported by Felschow *et al.* Therefore introduction of a photoactivatable group to this molecule holds great promise in the isolation of the PATr protein(s).

1. Azide

Substitution of the dimethylamino group in dansyl chloride by azide produces a photochemically reactive chemical group. The preparation of 1-azido-5-naphthalene sulfonyl chloride has been described (Muramoto, K., *Agric. Biol. Chem.*, 1984, 48 (11), 2695-2699), and it is also available commercially from Molecular Probes Inc. (Eugene, Oregon). Introduction of this compound into the synthetic scheme for DACS is straightforward and merely requires substitution for dansyl chloride.

This azido derivative, would enable isolation and characterization of the PATr protein(s), and would also find use as an irreversible, photoactivatable drug molecule.

2. Diaziridines

Substitution of a diaziridine group on the head group would accomplish many of the same goals as noted above.

3. Diazo Groups

Polyamine analogues with photoactivatible head groups are made using p-nitrophenyl 3-diazopyruvate, a reagent for introduction of a photoactivatable 3-diazopyruvate group to an aliphatic amine. This agent is also available from Molecular Probes, Inc. The desired derivative is made by reacting this reagent with the free amino, p-nitrophenyl activated linker precursor, purifying the linker/head group intermediate, and reacting it with the polyamine.

Analytical and Diagnostic Uses

The bispolyamine analogues and derivatives of the invention may also be used as reporter molecules and probes to assay other pharmacological targets, including soluble proteins, as described in PCT/US98/14896, which also describes the use of reporter head groups and polyamine transport assays.

TESTING INHIBITORS OF POLYAMINE TRANSPORT

Through screening bispolyamine compounds made by the various synthetic routes described above, several compounds were found to effectively inhibit polyamine transport. "R" values were calculated as the ratio of the IC_{50} in the absence of DFMO, or other polyamine synthesis inhibitor, over the IC_{50} in the presence of DFMO, or other polyamine synthesis inhibitor. An "R" value of 1 reflects a polyamine transport inhibitor that shows no change in the presence of a polyamine synthesis inhibitor, suggesting that the transport inhibitor fails to inhibit the transporter or is not specific for the transporter.

As expected, the presence of a polyamine synthesis inhibitor enhances the inhibition of cell growth by the bispolyamine transport inhibitors of the invention when used alone. A large enhancement reflects a good transport inhibitor that is specific for the polyamine transporter because it suggests that the transport inhibitor does not interact significantly with other cellular components. Preferred transport inhibitors of the invention will have "R" values of above about 2, but more preferably above about each of the following: 5, 10,

50, 100, 200, 300, and 400. Most preferred are compounds with "R" values of above about 500, above about 1000, or above about 10,000. Since significant "R" values may reflect conditions where neither the transport inhibitor nor the polyamine synthesis inhibitor alone are able to result in growth inhibition, the combination of the two may be considered to result in a synergistic effect, which varies according to the specificity of the transport inhibitor in combination with the specific synthesis inhibitor used. Such effects are not readily predictable in advance because the magnitude of inhibitory activity and degree of specificity are individual to each transport inhibitor.

The "R" values of the invention may also be considered in relation to the IC_{50} values of this invention's polyamine transport inhibitors in the presence or absence of a polyamine synthesis inhibitor. Such a consideration provides useful information regarding the potential usefulness of the transport inhibitor as an active ingredient. Preferred is a review of the "R" value versus the IC_{50} value in the presence of a polyamine synthesis inhibitor. This is useful because if that IC_{50} value is too high, the transport inhibitor is unlikely to be a viable active agent because of the necessary high concentrations needed for inhibitory activity. This requirement for a high concentration would not necessarily be negated even by very high "R" values. Thus inhibitors of the invention are preferably those that exhibit a IC_{50} value of about 100 μM or less when used in combination with a polyamine synthesis inhibitor. More preferable are inhibitors that exhibit IC_{50} values, in the presence of a polyamine synthesis inhibitor, of less than about each of the following: 75, 50, and 25 μM. Most preferred are compounds that exhibit IC_{50} values, in the presence of a polyamine synthesis inhibitor, of less than about 10, less than about 5, less than about 1, less than about 0.5, less than about 0.1, less than about 0.05, and less than about 0.01 μM.

Using both a kinetic measure and a biological assay, the present inventors observed high correlation between the inhibition of PAT and growth.

PHARMACEUTICAL AND THERAPEUTIC COMPOSITIONS

The bispolyamine analogues and derivatives of the invention, as well as the pharmaceutically acceptable salts thereof, may be formulated into pharmaceutical compositions. Pharmaceutically acceptable acid addition salts of the compounds of the invention which contain basic groups are formed where appropriate with strong or

moderately strong, non-toxic, organic or inorganic acids in the presence of the basic amine by methods known in the art. Exemplary of the acid addition salts that are included in this invention are maleate, fumarate, lactate, oxalate, methanesulfonate, ethanesulfonate, benzenesulfonate, tartrate, citrate, hydrochloride, hydrobromide, sulfate, phosphate and nitrate salts.

As stated above, the compounds of the invention possess the ability to inhibit PAT or polyamine synthesis, properties that are exploited in the treatment of any of a number of diseases or conditions, most notably cancer. A composition of this invention may be active *per se*, or may act as a "pro-drug" that is converted *in vivo* to active form.

The compounds of the invention, as well as the pharmaceutically acceptable salts thereof, may be incorporated into convenient dosage forms, such as capsules, impregnated wafers, tablets or injectable preparations. Solid or liquid pharmaceutically acceptable carriers may be employed. Pharmaceutical compositions designed for timed or delayed release may also be formulated.

Preferably, the compounds of the invention are administered systemically, *e.g.*, by injection. When used, injection may be by any known route, preferably intravenous, subcutaneous, intramuscular, intracranial or intraperitoneal. Injectables can be prepared in conventional forms, either as solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions.

Solid carriers include starch, lactose, calcium sulfate dihydrate, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate and stearic acid. Liquid carriers include syrup, peanut oil, olive oil, saline, water, dextrose, glycerol and the like. Similarly, the carrier or diluent may include any prolonged release material, such as glyceryl monostearate or glyceryl distearate, alone or with a wax. When a liquid carrier is used, the preparation may be in the form of a syrup, elixir, emulsion, soft gelatin capsule, liquid containing capsule, sterile injectable liquid (e.g., a solution), such as an ampoule, or an aqueous or nonaqueous liquid suspension. A summary of such pharmaceutical compositions may be found, for example, in *Remington's Pharmaceutical Sciences,* Mack Publishing Company, Easton Pennsylvania (Gennaro 18th ed. 1990).

The pharmaceutical preparations are made following conventional techniques of pharmaceutical chemistry involving such steps as mixing, granulating and compressing, when necessary for tablet forms, or mixing, filling and dissolving the ingredients, as appropriate, to give the desired products for oral or parenteral, including , topical, transdermal, intravaginal, intranasal, intrabronchial, intracranial, intraocular, intraaural and rectal administration. The pharmaceutical compositions may also contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and so forth.

Although the preferred routes of administration are systemic, the pharmaceutical composition may be administered topically or transdermally, e.g., as an ointment, cream or gel; orally; rectally; *e.g.*, as a suppository, parenterally, by injection or continuously by infusion; intravaginally; intranasally; intrabronchially; intracranially intra-aurally; or intraocularly.

For topical application, the compound may be incorporated into topically applied vehicles such as a salve or ointment. The carrier for the active ingredient may be either in sprayable or nonsprayable form. Non-sprayable forms can be semi-solid or solid forms comprising a carrier indigenous to topical application and having a dynamic viscosity preferably greater than that of water. Suitable formulations include, but are not limited to, solution, suspensions, emulsions, creams, ointments, powders, liniments, salves, and the like. If desired, these may be sterilized or mixed with auxiliary agents, e.g., preservatives, stabilizers, wetting agents, buffers, or salts for influencing osmotic pressure and the like. Preferred vehicles for non-sprayable topical preparations include ointment bases, e.g., polyethylene glycol-1000 (PEG-1000); conventional creams such as HEB cream; gels; as well as petroleum jelly and the like.

Also suitable for topical application are sprayable aerosol preparations wherein the compound, preferably in combination with a solid or liquid inert carrier material, is packaged in a squeeze bottle or in admixture with a pressurized volatile, normally gaseous propellant. The aerosol preparations can contain solvents, buffers, surfactants, perfumes, and/or antioxidants in addition to the compounds of the invention.

For the preferred topical applications, especially for humans, it is preferred to administer an effective amount of the compound to a target area, *e.g.*, skin surface, mucous

membrane, eyes, *etc.* This amount will generally range from about 0.001 mg to about 1 g per application, depending upon the area to be treated, the severity of the symptoms, and the nature of the topical vehicle employed.

The compositions of the invention be given in combination with one or more additional compounds that are used to treat the disease or condition. For treating cancer, the polyamine analogues and derivatives are given in combination with anti-tumor agents, such as mitotic inhibitors, *e.g.*, vinblastine; alkylating agents, *e.g.*, cyclophosphamide; folate inhibitors, *e.g.*, methotrexate, pritrexim or trimetrexate; antimetabolites, *e.g.*, 5-fluorouracil and cytosine arabinoside; intercalating antibiotics, *e.g.*, adriamycin and bleomycin; enzymes or enzyme inhibitors, *e.g.*, asparaginase; topoisomerase inhibitors, *e.g.*, etoposide; or biological response modifiers, *e.g.*, interferon. In fact, pharmaceutical compositions comprising any known cancer therapeutic in combination with the polyamine analogues and derivatives disclosed herein are within the scope of this invention. Most preferably, the present compounds are administered in combination with a polyamine synthesis inhibitor such as DFMO.

The pharmaceutical compositions of the invention may also comprise one or more other medicaments such as anti-infectives including antibacterial, anti-fungal, anti-parasitic, anti-viral, and anti-coccidial agents.

Typical single dosages of the compounds of this invention are between about 1 ng and about 10 g/kg body weight. The dose is preferably between about 0.01 mg and about 1g/kg body wt. and, most preferably, between about 0.1 mg and about 100 mg/kg body wt. For topical administration, dosages in the range of about 0.01-20% concentration of the compound, preferably 1-5%, are suggested. A total daily dosage in the range of about 1-500 mg is preferred for oral administration. The foregoing ranges are, however, suggestive, as the number of variables in regard to an individual treatment regime is large, and considerable excursions from these recommended values are expected and may be routinely made by those skilled in the art.

Effective amounts or doses of the compound for treating a disease or condition can be determined using recognized *in vitro* systems or *in vivo* animal models for the particular disease or condition. In the case of cancer, many art-recognized models are known and are representative of a broad spectrum of human tumors. The compounds may be tested for

inhibition of tumor cell growth in culture using standard assays with any of a multitude of tumor cell lines of human or nonhuman animal origin. Many of these approaches, including animal models, are described in detail in Geran, R.I. *et al.*, "Protocols for Screening Chemical Agents and Natural Products Against Animal Tumors and Other Biological Systems (Third Edition)", *Canc. Chemother. Reports*, Part 3, 3:1-112.

Synthetic Methods

The synthetic methods necessary to produce the polyamine analogues and derivatives for the preparation of bispolyamines of the invention, including parallel library synthesis and combinatorial approaches, have been described in PCT/US98/14896.

Additionally, this invention provides synthetic methods whereby bispolyamines may be readily produced (see Figures 3A and 3B as well as examples below). Briefly, the method uses ^{t}Boc protected polyamine derivatives as starting substrates that are linked to form bispolyamines. These bispolyamine products are then purified by ion exchange chromatography. Elution of the products permits recovery and availability for optional subsequent deprotection.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

EXAMPLE I

Screening of Polyamine Analogues in Transport and Growth Assays

The effect of a number of potential PAT transport inhibitors on PAT and growth of MDA cells is summarized in Figure 2 (3-98). The ratio "R" is the IC_{50} for polyamine alone relative to the IC_{50} for the polyamine analogue combined with an ODC inhibitor. This value of "R", indicates the relative level of "synergism" between the polyamine analogue and ODC inhibitor. Under the growth assay conditions, the ODC inhibitor alone shows no inhibition.

EXAMPLE II

Ki determinations and structure activity relationships

The bispolyamine analogues and derivatives of the invention may be evaluated for their ability to inhibit the uptake of spermidine into MDA cells in culture. Joro spider toxin JSTx-3 is available from Calbiochem; 1-Naphthylacetylspermine is available from RBI. Deoxyspergualin was a generous gift from Paul Gladstone. K_is were measured for the bispolyamine analogues in Figure 16 and the results are shown therein.

EXAMPLE III

IC_{50} against MDA cells with DFMO and spermidine

A cellular assay was developed to highlight the ability of the amino acid/spermine amides to work in concert with the ODC inhibitor DFMO in the presence of added 1 μM spermidine. In this assay, no growth inhibition is observed with DFMO alone because the cells are able to utilize the spermidine added to the culture media even when polyamine biosynthesis is inhibited. Thus inhibition of uptake of the exogenously added spermidine by any of the tested analogues or derivatives results in observable growth inhibition due to polyamine depletion.

Results with some deprotected bispolyamines are shown in Figure 16.

EXAMPLE IV

Synthesis of bispolyamines

The substrates for synthesizing bispolyamines may be prepared by a two part process: synthesis of a linker moiety and synthesis of monoprotected polyamines.

Exemplary linkers are prepared by converting the corresponding acid chlorides to p-nitrophenyl activated esters using 4-nitrophenol. See Figure 4. Examples of such activated esters are shown in Figure 18. These are purified by recrystallization in $EtOH/CH_2Cl_2$ (10-30% EtOH) and dried under high vacuum.

Polyamines may be protected by methods well known in the art. For example, spermine (3 equivalents or "eq") is monoprotected with di-tert-butyldicarbonate (1 eq) which is slowly added over a 1.5 hour time frame to a solution of spermine in

dioxane/water with NaOH (1 eq). See Figure 5. After stirring for 24 hours, the solvent is evaporated and the compound purified over a Bio-Rex cation exchange column (45x 2.5 cm).

Synthesis of bispolyamines may be by the reaction scheme shown in Figure 3A, where p-nitrophenyl activated esters are reacted with protected spermines.

For example, to a flask of 2.2 equivalents of N^1-^{t}Boc-spermine in 10 mL methanol, 1 equivalent of 4-nitrophenyl ester dissolved in 5 mL DMF and 10 mL of MeOH was added drop by drop with stirring for three hours or overnight.

A second equivalent of the 4-nitrophenyl ester may be added as a solid and allow to stir for an additional 3 hours. The solvent was evaporated and the DMF removed under high vacuum. The crude product was originally redissolved in water and purified over a Bio-Rex cation exchange column (45 x 2.5 cm).

Optionally, 50% MeOH/water may be used as the solvent for improved solubility of the bispolyamine. The compounds were eluted with a gradient ranging from 0 through 1 - 2 N NH_4OH. The appropriate fractions were pooled and the solvent evaporated to produce the bispolymer.

N^1-^{t}Boc-spermine has been coupled to the eight paranitrophenyl esters shown in Figure 18, including the succinyl (n = 2) linked dispermine. I have purified six of these compounds using Bio-Rex cation exchange chromatography. The crude product is generally a mixture consisting of two spots when analyzed on thin layer chromatography (TLC). Both spots migrate higher than N^1-^{t}Boc-spermine with one generally migrating close to the solvent front and the other migrating somewhat higher than N^1-^{t}Boc-spermine but this varies with the p-nitrophenyl ester.

In general, this spot migrates higher as n increases in value. In the case where n = 10 (dodecanedioyl derivative) the two spots migrate very close to each other near the solvent front. The purification of compounds with greater n values are generally eluted with a 0 to 1 or 1.5 N NH_4OH gradient.

One exception, however, is the succinyl derivative which consists of four reaction products on TLC. This derivative was successfully purified using 50% MeOH/water instead of water as solvent.

For removal of the N^1-^{t}Boc protecting group, 5 mL of 3M HCl may be added to the above reaction conditions followed by stirring for one hour.

^{1}H and ^{13}C NMR spectra for the t-boc protected bispolyamines have been completed except for ORI 1268 where only a ^{1}H was completed. Likewise, ^{1}H and ^{13}C NMR spectra have also been obtained for the deprotected final products ORI 1236, 1288, 1289, 1290. Mass spec analysis has been completed for ORI 1288 and ORI 1290.

EXAMPLE V

Polyamine transport inhibition by bispolyamines

Most of the spermine dimers that have been tested provided very good K_i for transport inhibition with values under 75 nM. ORI 1236 was the most potent inhibitor with a K_i of 22 nM. This value is comparable with the K_i for ORI 1090 (between 10- 22 nM for MDA cells). .Only ORI 1275 had a K_i that was above 100 nM (K_i = 219 nM). The results were generally mirrored in the growth inhibition assay. All of the compounds where synergistic with DFMO with IC_{50}s of 10 μM or less. The most potent growth inhibitor was ORI 1288 followed by ORI 1286 > 1236 > 1289 > 1290 > 1275 > 1299.

Without being bound by theory, it appears that shorter linked spermine dimers are slightly more potent than the longer chained analogs. Because there was not a more dramatic difference in activity between the analogs, it is suggested that there is a fairly large degree of tolerance for the length of the aliphatic linker in a transporter's polyamine binding site. This also supports the finding that there is some leeway in linker length of PTI inhibitors such as 1202 and 1090. These bispolyamine molecules may interact in similar fashion with respect to the transporter as ORI 1202 and ORI 1090.

It was observed that ORI 1236 in combination with DFMO gave a maximum growth inhibition that was less than the ORI 1202/DFMO control. This suggested that ORI 1236 may partially rescue from polyamine depletion. Subsequently, ORI 1236 was found to partially rescue from DFMO. Almost all other bispolyamine compounds tested except for ORI 1287 gave a maximum growth inhibition that was less than the ORI 1202/DFMO control. ORI 1236 and 1290 have been re-tested and only ORI 1290 displayed rescue

All references cited herein are hereby incorporated by reference in their entireties, whether previously specifically incorporated or not.

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features herein before set forth as follows in the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A polyamine analogue or derivative that binds to a polyamine-binding site of a molecule and/or inhibits polyamine transport, which analogue or derivative is a bispolyamine.

2. An analogue or derivative according to claim 1 wherein said bispolyamine comprises at least one N^1-monosubstituted polyamine that is an N^1-monosubstituted putrescine, spermidine, or spermine.

3. An analogue or derivative according to claim 2 wherein said N^1-monosubstitution comprises an amide linkage.

4. An analogue or derivative according to claim 2 wherein said N^1-monosubstitution comprises a sulfonamide linkage.

5. An analogue or derivative according to claim 2 wherein said N^1-monosubstitution comprises an amine.

6. An analogue or derivative according to claim 3 wherein said N^1-monosubstitution further comprises a linker moiety.

7. An analogue or derivative according to claim 3 wherein said N^1-monosubstitution further comprises an amino alkyl moiety.

8. An analogue or derivative according to claim 3 wherein said N^1-monosubstitution further comprises an amino acid head group or derivative thereof.

9. An analogue or derivative according to claim 8 wherein said amino acid head group is protected, a naturally occurring amino acid, or a non-naturally occurring amino acid.

10. A polyamine analogue or derivative according to claim 1 wherein said analogue or derivative is selected from the compounds listed in Figure 16.

11. A polyamine analogue or derivative according to claim 1 wherein said analogue or derivative is selected from the compounds listed in Figure 17.

12. An analogue or derivative according to claim 4 wherein said N^1-monosubstituted polyamine is selected from the compounds listed in Figure 9h.

13. An analogue or derivative according to claim 3 wherein said N^1-monosubstituted polyamine is selected from the compounds listed in Figures 9a-9c.

14. An analogue or derivative according to claim 8 wherein said N^1-monosubstituted polyamine is selected from the compounds listed in Figures 9d-9g.

15. An analogue or derivative according to claim 11 wherein said N^1-monosubstituted polyamine is selected from the compounds listed in Figures 9a-9f.

16. An analogue or derivative according to claim 1 wherein said analogue or derivative further comprises a reactive moiety that is capable of forming covalent bonds with a nucleophilic site on a target molecule.

17. An analogue or derivative according to claim 16, wherein said target molecule is a protein or a nucleic acid.

18. An analogue or derivative according to claim 16, wherein said target molecule is a cellular receptor or other cell surface molecule.

19. A composition useful for treating a disease or condition in which the inhibition of polyamine transport is desirable, comprising

a polyamine analogue or derivative according to any one of the preceding claims and

a pharmaceutically acceptable excipient.

20. A composition useful for treating a disease or condition in which the inhibition of polyamine transport and synthesis is desirable, comprising the composition of claim 19 and an inhibitor of polyamine synthesis.

21. A composition according to claim 20 wherein said inhibitor of polyamine synthesis is difluoromethylornithine (DFMO).

22. A composition according to claim 20, further comprising, in combination with said composition, one or more additional agents known to be useful for treating said disease or condition

23. A method for treating a disease or a condition in a subject associated with undesired cell proliferation and/or which is treatable by inhibition of polyamine transport, comprising adminstering to said subject an effective amount of a polyamine analogue or derivative of any one of claims 1-18.

24. A method according to claim 23 wherein said undesired cell proliferation is associated with proliferation of cells of the immune system, cell of the vascular neontima, tumor cells or with undesired angiogenesis.

25. A method according to claim 23 wherein said disease or condition is cancer or post-angioplasty injury.

26. A method for treating a disease or a condition in a subject associated with undesired cell proliferation and/or which is treatable by inhibition of polyamine transport and synthesis, comprising adminstering to said subject an effective amount of a polyamine analogue or derivative according to any one of claims 1-18, and an inhibitor of polyamine synthesis.

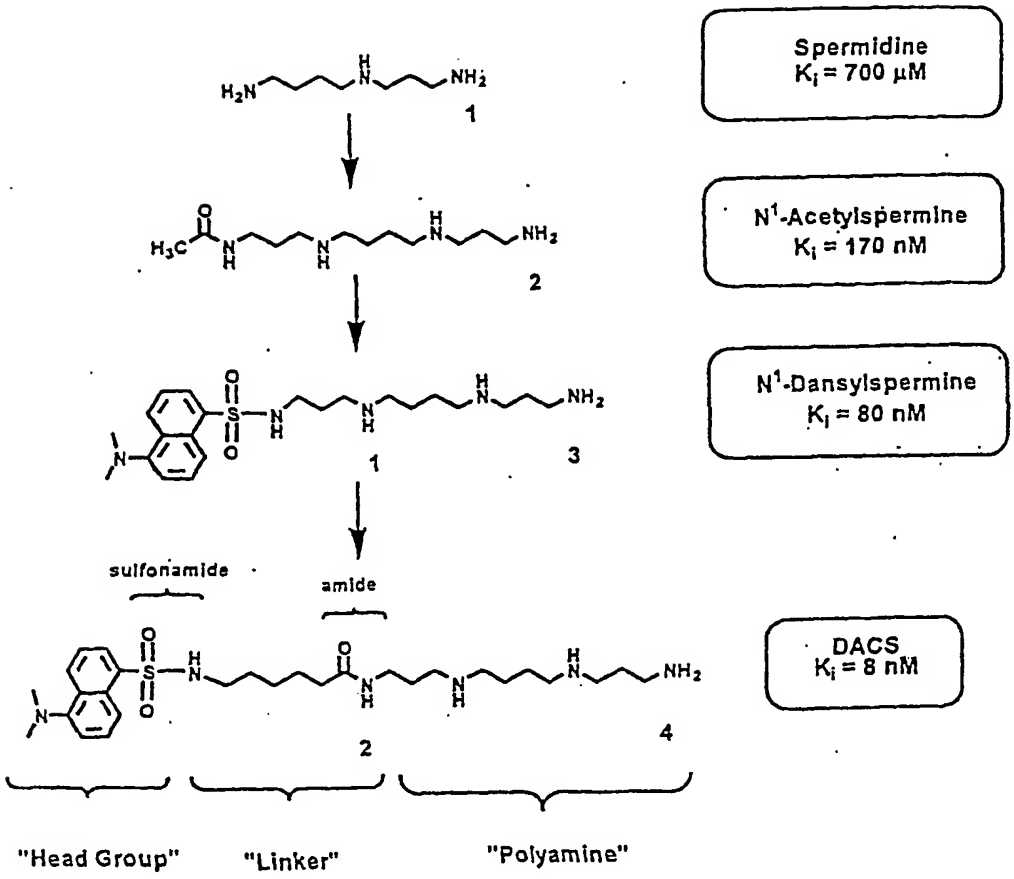
27. A method according to claim 26 wherein said inhibitor of polyamine synthesis is difluoromethylornithine (DFMO).

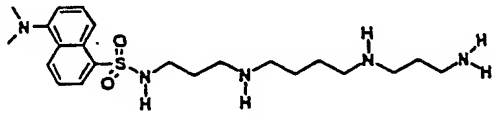
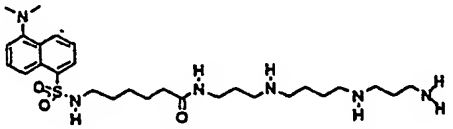
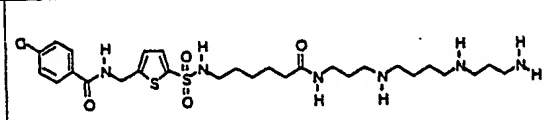
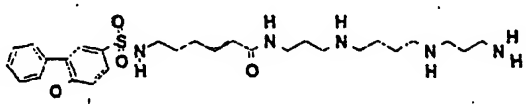
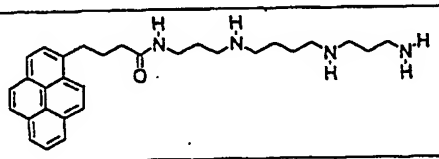
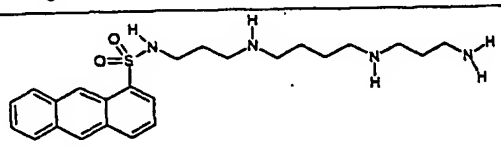
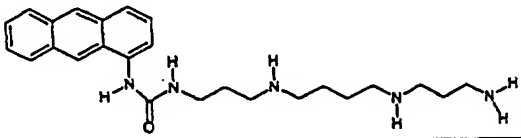
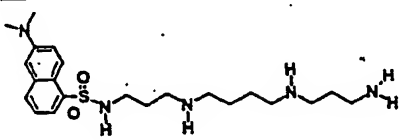
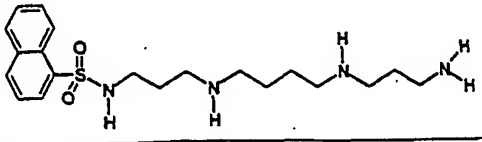
28. A method according to claim 26, further comprising one or more additional agents known to be useful for treating said disease or condition.

29. A method according to any of claims 23-28 wherein said administering is performed orally, parenterally, topically, transdermally, intravaginally, intranasally, intrabronchially, intracranially, intraocularly, intraaurally, or rectally, or by injection.

30. A method according to claim 29 wherein said administering by injection is intravenous, subcutaneous, intramuscular, intracranial, or intraperitoneal.

Fig. 1


Spermidine
K_i = 700 µM
N^1-Acetylspermine
K_i = 170 nM
N^1-Dansylspermine
K_i = 80 nM
DACS
K_i = 8 nM
sulfonamide
amide
"Head Group"
"Linker"
"Polyamine"

#	Structure	Ki (M)[a]	R[b]	Method[c]
3		0.080	20	I
4		0.010	400	IX, XIII
5		0.010	210	XIII
6		0.005	220	XIII
7		0.10	3.6	III
8		0.110	3.7	II
9		0.440	2.7	IV
10		0.050	>10	XV
11		0.190	2.4	XV

a Inhibition of polyamine uptake: Ki determined from Lineweaver-Burke double reciprocal plots
b Inhibition of Tumor Cell Growth: R is ratio of IC50 (compound alone) to IC50 (compound + DFMO)
c Numbers refer to Examples (describing synthesis)
d Purchased from Aldrich Chemical Company

Fig. 2/1

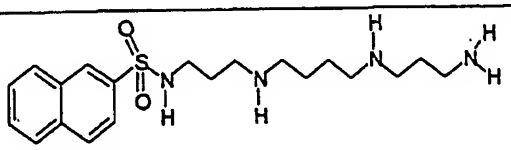
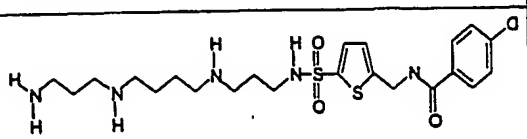
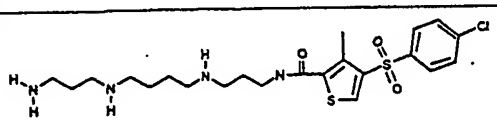
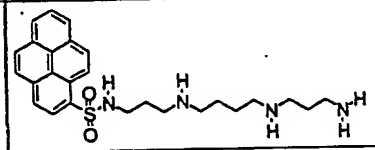
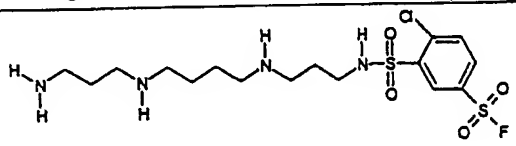
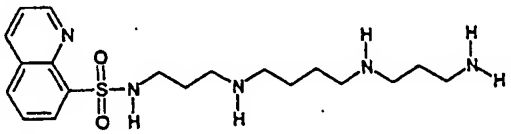
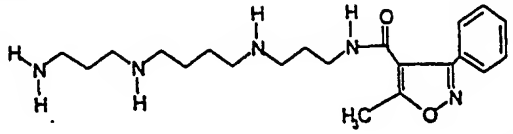
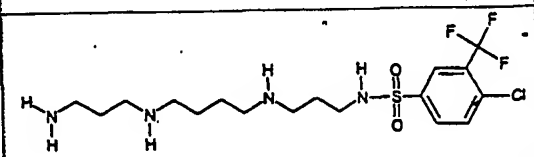
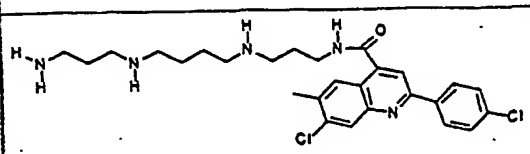
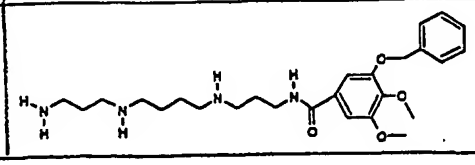
#	Structure	Ki (M)[a]	R[b]	Method[c]
12		0.150	4.3	XV
13		0.058	>47	XV
14		0.037	14	XVII
15		0.091	2.2	II
16		0.08	2.1	XV
17		0.43	>31	XV
18		0.083	40	XVII
19		0.24	>10	XV
20		0.28	1.0	XVII
21		0.084	1.0	XVII

Fig. 2/2

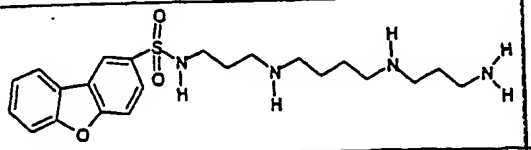
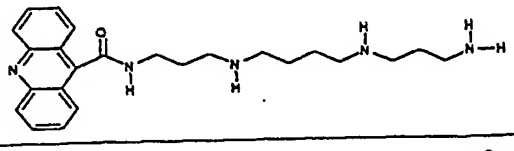
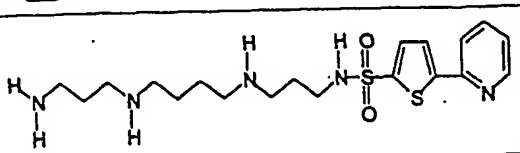
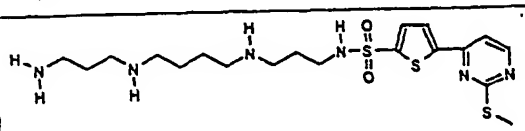
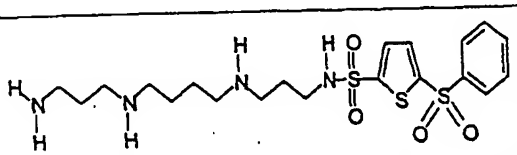
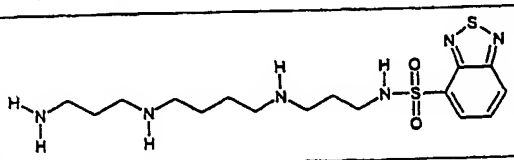
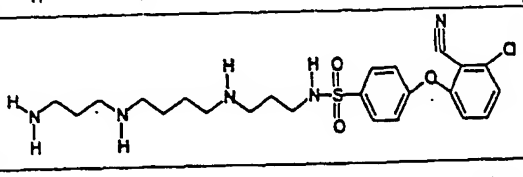
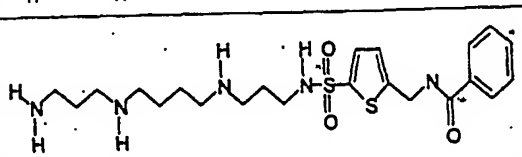
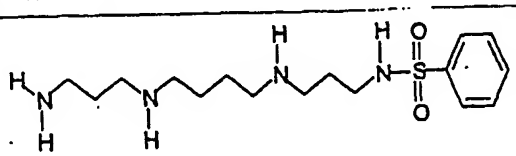
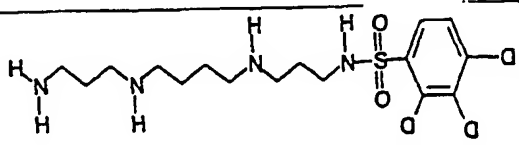
#	Structure	Ki (M)[a]	R[b]	Method[c]
22		0.066	11	XV
23		0.250	6.2	II
24		0.23	10	XV
25		0.067	8.6	XV
26		0.180	15	XV
27		0.650	9.9	XV
28		0.054	9.3	XV
29		0.076	>46	XV
30		0.120	>10	XV
31		0.083	>12	XII

Fig. 2/3

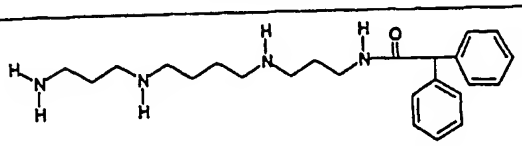
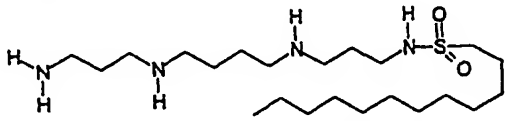
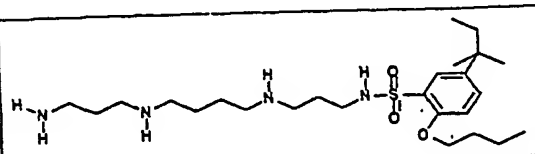
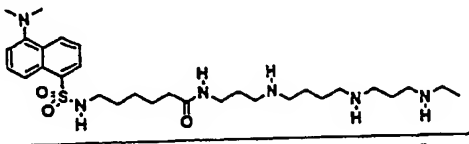
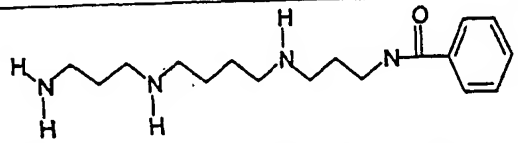
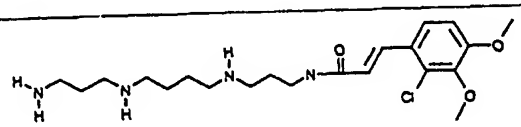
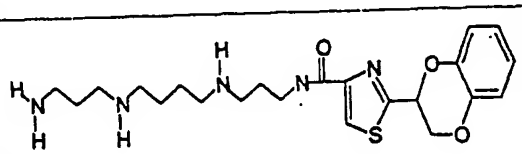
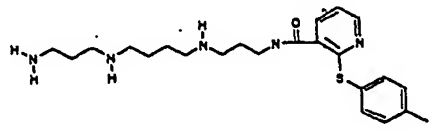
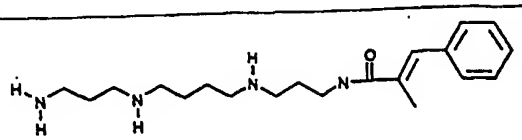
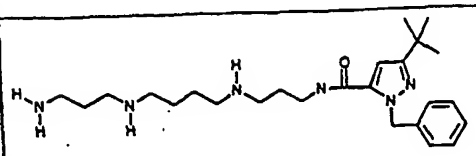
#	Structure	Ki (M)[a]	R[b]	Method[c]
32		0.093	2.1	XVII
33		0.17	1.4	XV
34		0.120	1.0	XV
35		0.041	33	XIII
36		0.61	>2	XVII
37		0.150	2.4	XVII
38		0.140	1.0	XVII
39		0.500	1	XVII
40		0.086	18	XVII
41		0.200	1.0	XVII

Fig. 2/4

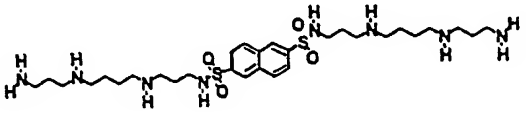
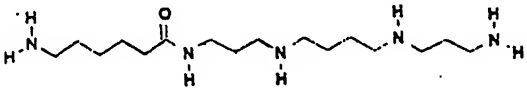
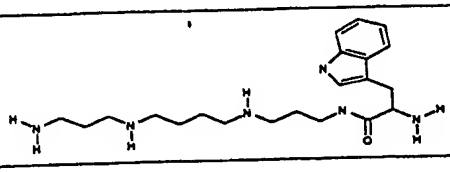
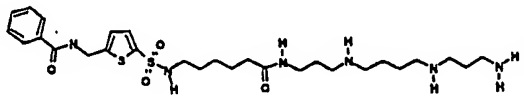
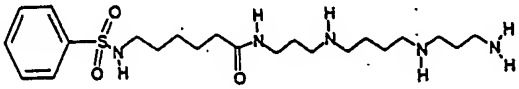
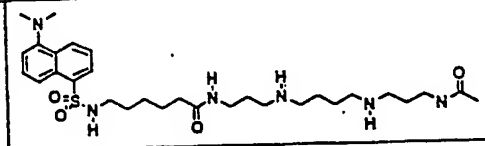
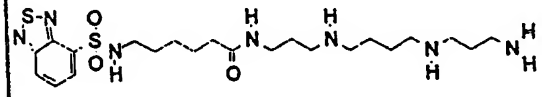
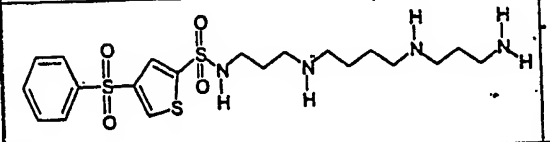
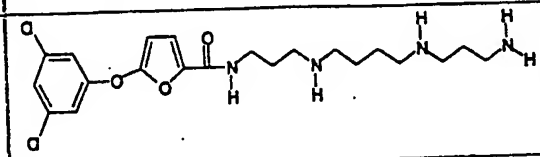
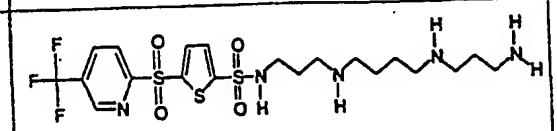
#	Structure	Ki (M)[a]	R[b]	Method[c]
42		0.110	1.1	XIV
43		0.033	76	XVII
44		0.073	39	XIII
45		0.052	3.0	XIII
46		0.082	63	XIII
47		2.1	6.8	XIII
48		0.079	>49	XIII
49		0.067	3.2	XV
50		0.12	1.0	XVII
51		0.083	1.5	XV

Fig. 2/5

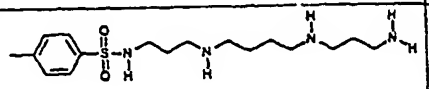
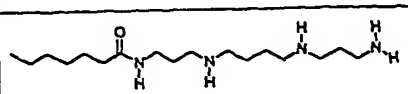
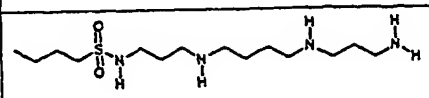
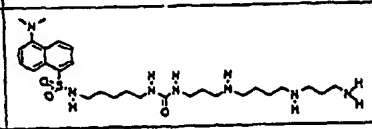
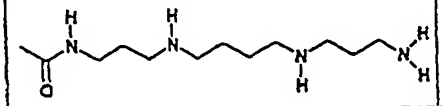
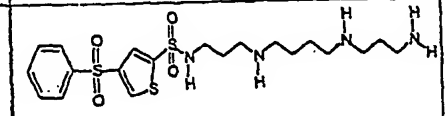
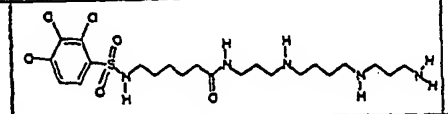
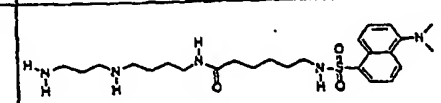
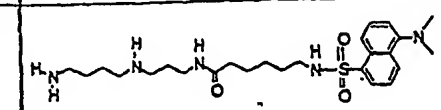
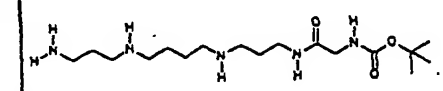
#	Structure	Ki (M)[a]	R[b]	Method[c]
52		0.094	5.3	XV
53		0.18	1.0	XV
54		0.19	2.0	XV
55		0.079	>1.1	IV
56		0.190		d
57		0.017	170	XV
58		0.050	189	XIII
59			>1	XIII
60			>1	XIII
61		0.200	1.0	XIII

Fig. 2/6

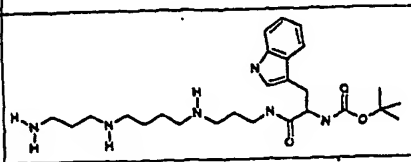
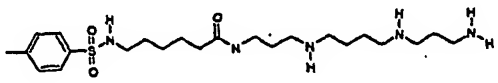
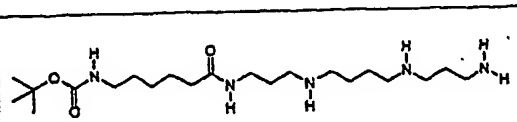
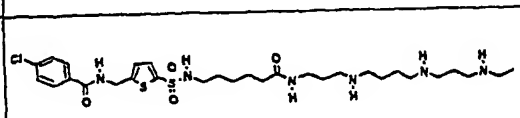
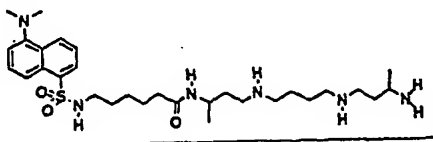
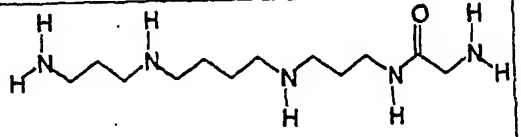
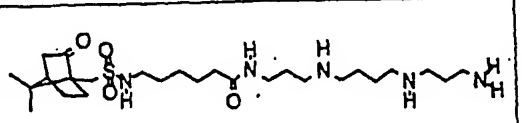
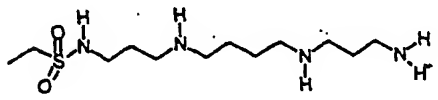
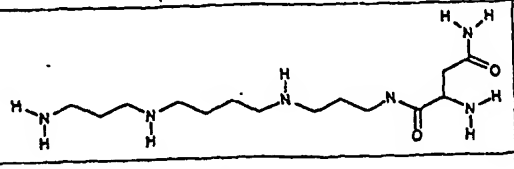
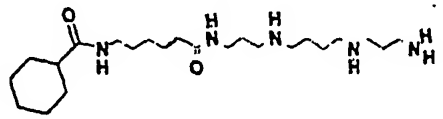
#	Structure	Ki (M)[a]	R[b]	Method[c]
62			>2.0	XIII
63		0.050	>1	XIII
64		0.046		XIII
65		0.012		XIII
66		0.018	27	XIII
67		0.07	1.0	XIII
68		0.110	>4.4	XIII
69		0.22	1	XV
70		0.033	>12.2	XIII
71		0.160	>1.5	XIII

Fig. 2/7

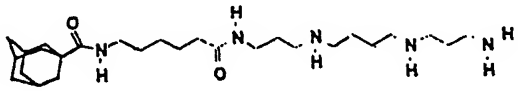
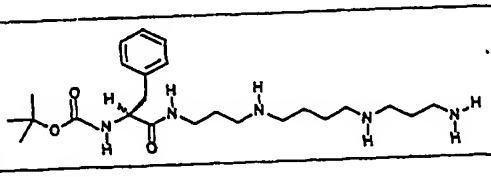
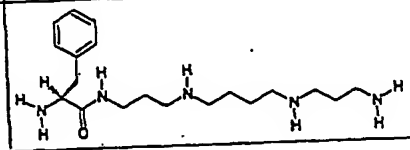
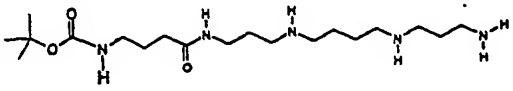
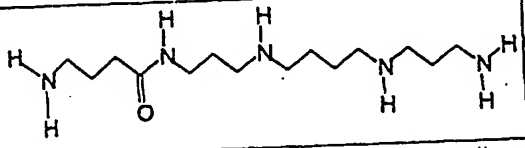
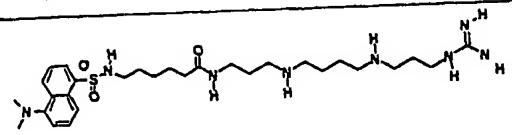
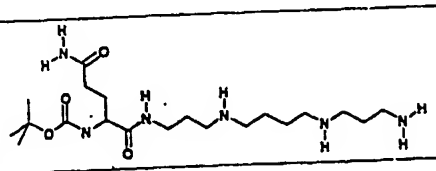
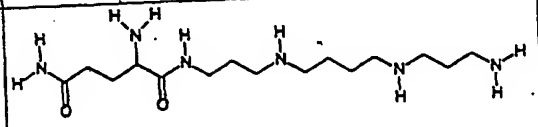
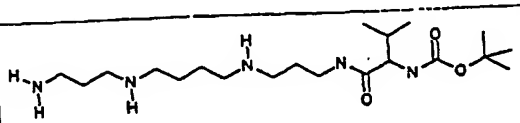
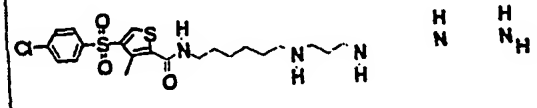
#	Structure	Ki (M)[a]	R[b]	Method[c]
72		0.031	>100	XIII
73		0.094	>1	XIII
74		0.200	1.0	XIII
75		0.130	>1	XIII
76		0.040	1.0	XIII
77		0.093	1	XIII
78		0.156		XIII
79		0.047	1	XIII
80		0.258		XIII
81		0.0096	153	XIII

Fig. 2/8

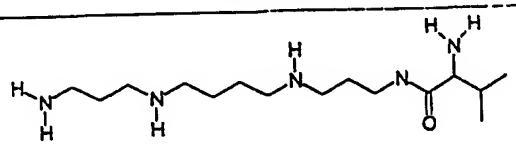
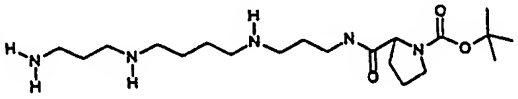
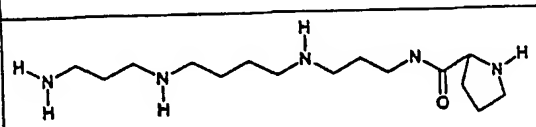
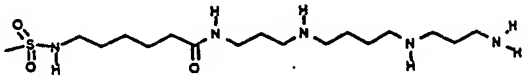
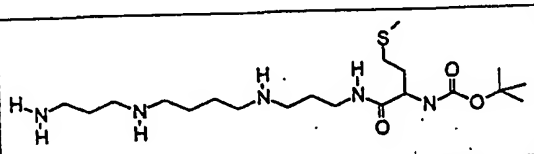
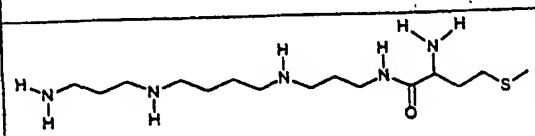
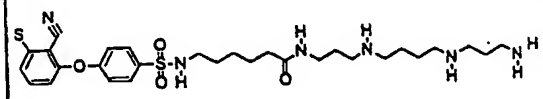
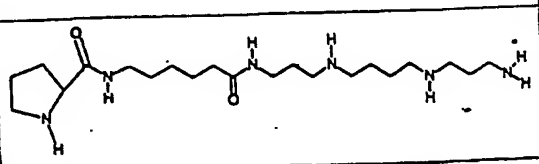
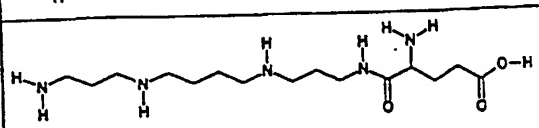
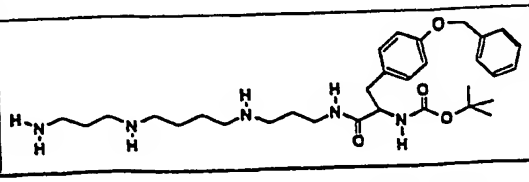
#	Structure	Ki (M)[a]	R[b]	Method[c]
82		0.097	>54	XIII
83		0.183		XIII
84		0.036	>3.2	XIII
85		0.048	>6.5	XIII
86		0.091		XIII
87		0.034	>1	XIII
88		0.014	>40	XIII
89		0.020	>1	XIII
90		0.077		XIII
91		0.037	1	XIII

Fig. 2/9

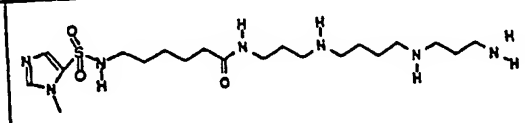
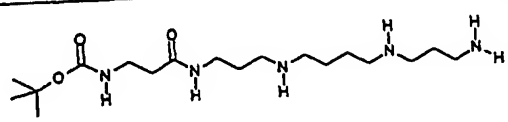
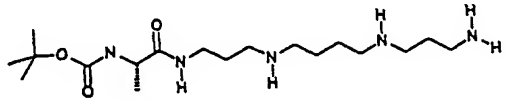
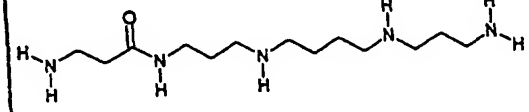
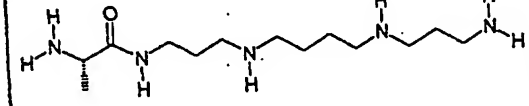
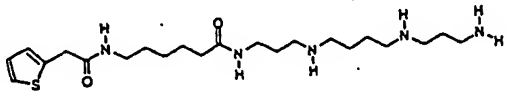
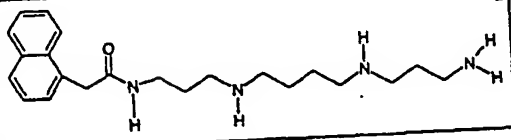
#	Structure	Ki (M)[a]	R[b]	Method[c]
92		0.300	1	XIII
93		0.061	1	XIII
94		0.042	1	XIII
95		0.050	1	XIII
96		0.034	1	XIII
97		0.027	1	XIII
98		0.180	12	d

Fig. 2/10

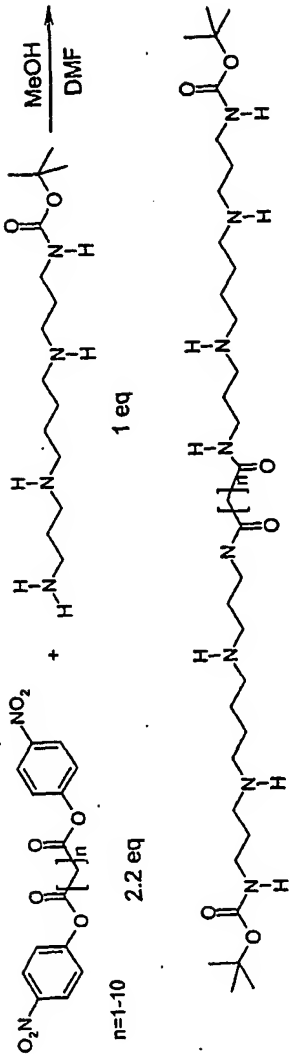


Fig. 3A

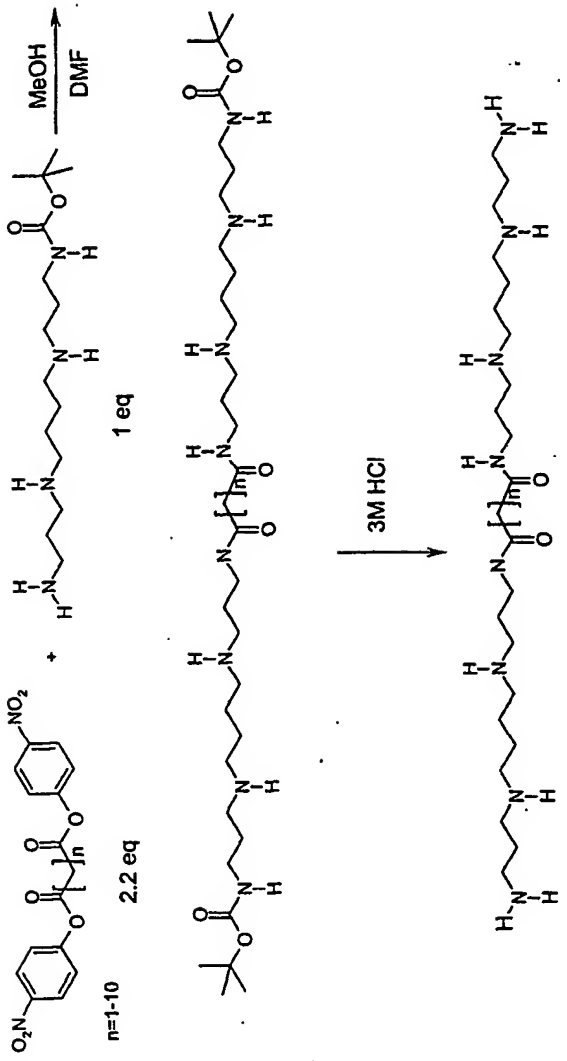


Fig. 3B

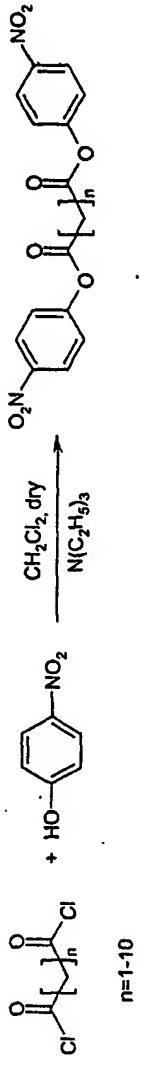

NO2
O
n
O
O
O2N
CH2Cl2, dry
N(C2H5)3
+ HO
NO2
O
Cl
n
O
Cl
n=1-10

Fig. 4

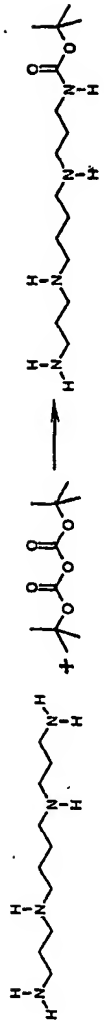
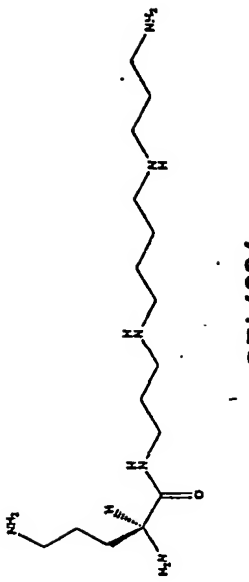
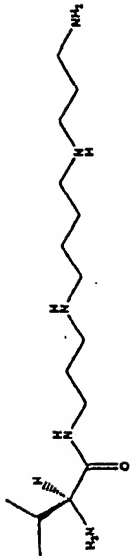

O
O
O
O
O
O
+
H
N
H
N
H
N
H
N
H
N
H
N
H
N
H
N
H

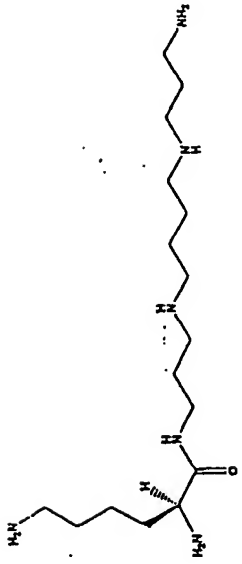
Fig. 5



ORI 1224
L-Orn-Spm



ORI 1157
L-Val-Spm



ORI 1202
L-Lys-Spm

Fig. 6

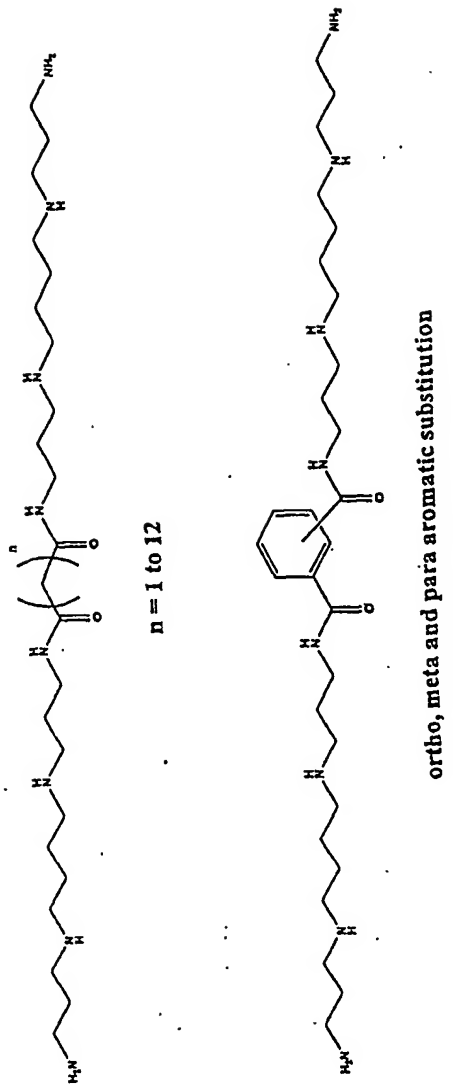
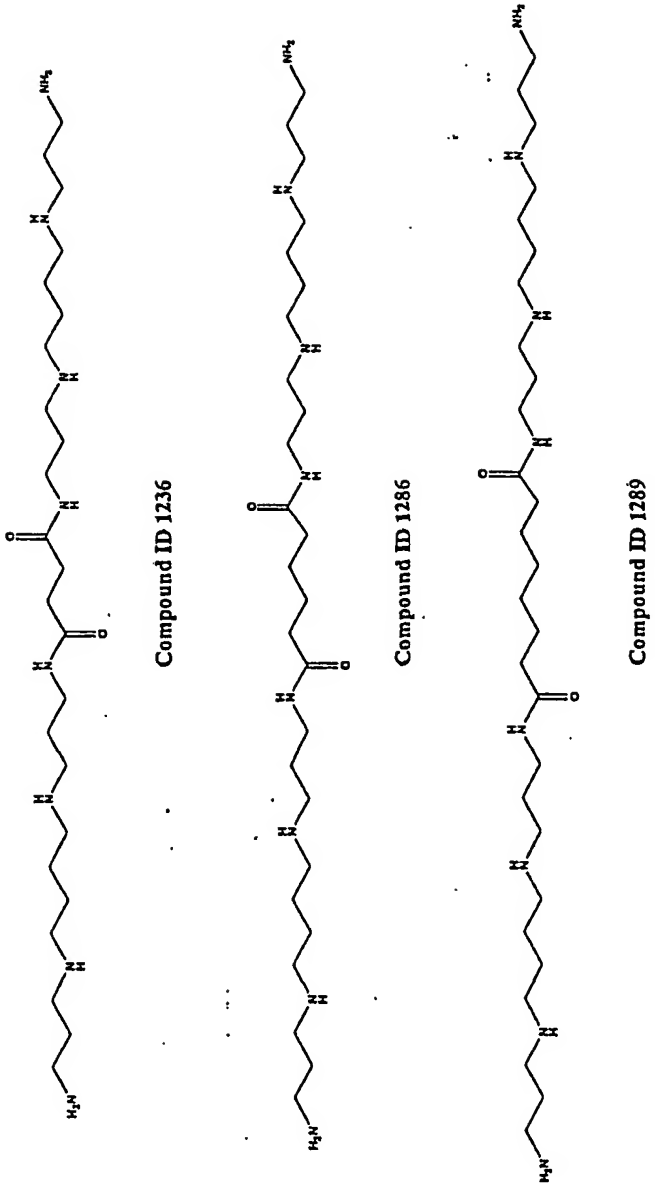


Fig. 7



Compound ID 1236

Compound ID 1286

Compound ID 1289

Fig. 8

Fig. 9A

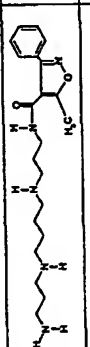
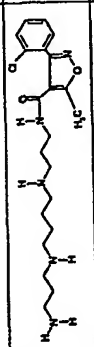
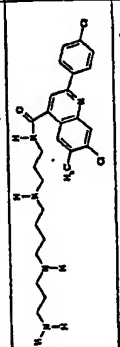
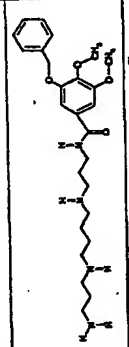
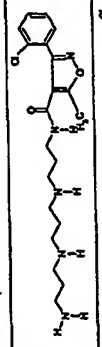
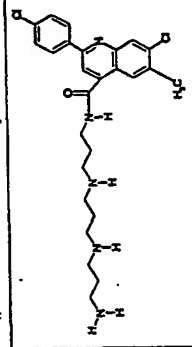
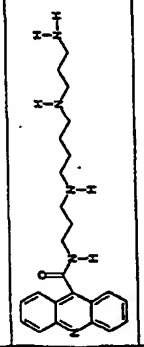
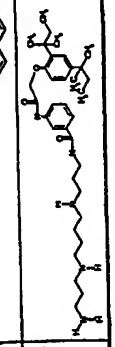
N1-monosubstituted polyamines: amides, no linker								
ID	mol weight	Structure	Transport>Cell Line	Ki	Growth Inhibtion>Cell Line	Half Effect Drug DFMO	IC50	
1032	387.5295		MDA	0.19	MDA	3.58	>300	
			MDA	0.083				
1033	421.9745		MDA	1.0	MDA		>300	
1035	516.5189		MDA	0.28	MDA		50	
1037	472.6331		MDA	0.084	MDA		100	
1038	407.9474		MDA	>10	MDA		>300	
1039	502.4918		MDA	>10	MDA		30	
1043	407.5635		MDA	0.344*	MDA	22.3	200	
			MDA	0.4				
1053	394.5648		MDA	0.54	MDA		260	
1072	595.8762		mda	>1				

Fig. 9A (cont'd)

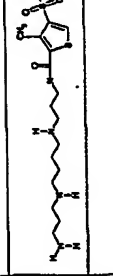
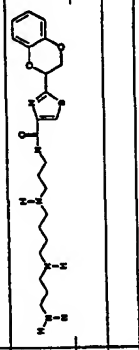
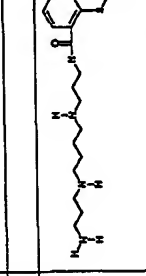
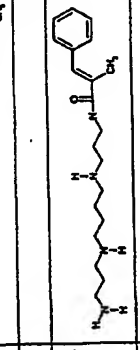
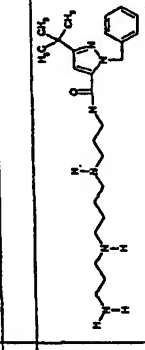
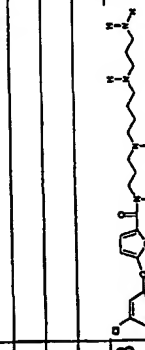
1073	306.4549		MDA	MDA	0.61	mda	150	>300
1076	426.9911		MDA	MDA	0.116*	mda	28.1	150
			MDA	MDA	0.165*	mda	2.46	56
1077	501.1143		MDA	MDA	0.11*	mda		
			MDA	MDA	0.037	mda		19
1078	447.604		MDA	MDA	0.19*	mda		19.4
						pc-3		24.4
						caco-2		6.9
						cem		83
1079	429.6323		MDA	MDA	0.594*	pc-3		
						mda		78
1080	346.5202		MDA	MDA	0.062*	mda	7.4	190
			MDA	MDA	0.086	mda		26
1081	442.6531		MDA	MDA	0.297*	mda		
						pc-3		5.5
						caco-2		23.0
						cem		1.7
1104	457.4043		MDA	MDA	0.12	mda		18
						pc-3		20.2
						caco-2		36.2
						cem		4.5

Fig. 9A (cont'd)

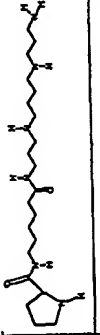
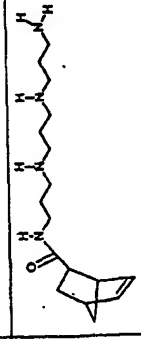
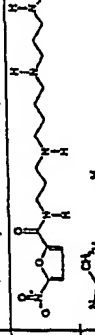
1163	302.4638		MDA	0.083				>100
1166	230.36				mda			>100
					H157			
1167	256.3943				mda			>100
					h157			
1169	412.62		MDA	0.0252	mda	>300	>300	>300
					pc-3	20.1		
1208	308.47							
1210	352.57							
1211	341.41							
1213	328.4829							
1214	325.46							
1215	284.45							
1216	313.49							

Fig. 9A (cont'd)

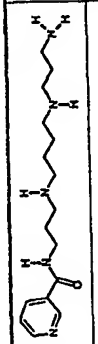
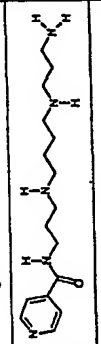
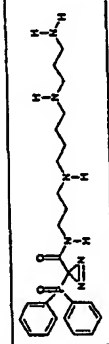
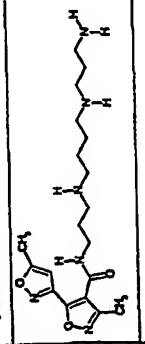
1217	307.44		MDA								
1218	307.4424				1.14						
1235	364.5792					mda	>300				
1240	378.6062							pc-3	>300		
1249	470.5594										
1251	392.5053		MDA		> 1						
1347	472.6795										

Fig. 9B

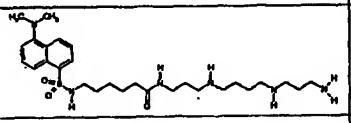
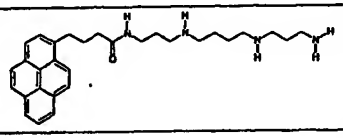
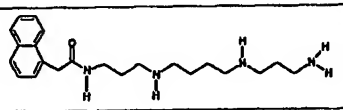
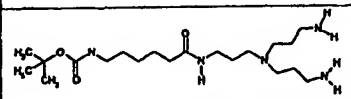
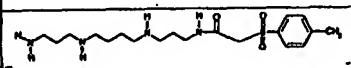
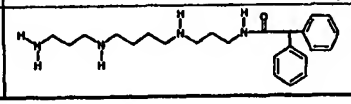
N1-monosubstituted polyamines: amides, with linker							
ID	mol weight	Structure	Transport>Cell Line	Ki	Growth Inhibtion>Cell Line	Half Effect Drug DFMO	IC50
1002	548.7972		MDA-MB-231	.024*	MDA-MB-231	2.2	>100
			A172	.016*			
			PC-3	0.0339*			
			MCF-7	0.012			
			MDA	0.0152*			
			CaCo	0.0078*			
			mda	0.0245-0.13	MDA-MB-231	2.0	>100
			mda	0.0052-0.03	mda	0.63	450
			MDA	8.6 nM	mda	2.0	380
					mcf-7		72
					casmc		
1009	472.6795		MDA	0.104	MDA	<3	25
			A172	0.12			
					MDA	9.4	79
1022	370.5425		MDA	0.230	MDA	8.26	>300
1040	401.5974				mda		>100
1055	398.5718		MDA		mda		6.9
1056	396.5807		MDA	0.11*	MDA		150

Fig. 9B (cont'd)

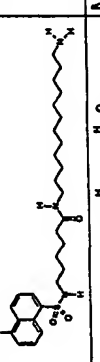
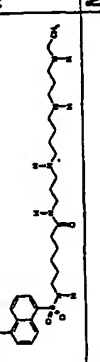
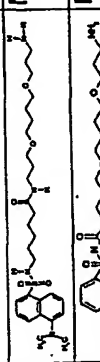
1059	546.822		MDA	6.5*	mda		70
						>300	>300
1060	439.8164		MDA	0.099	mda		
						<3	360
1061	576.8513		MDA	0.00895	mda		
			MDA	0.0942		9.81	560
			MDA	41.2 nM.	mda		
			MDA	57.8 nM			18
1063	550.7666		MDA	88*	mda		
						>100	>100
1064	510.7013		MDA	> 30	mda		
						>30	>30
1065	632.9597		MDA	0.76	mda		
						27	27
1066	650.9722		MDA	19.2*	mda		
					pc-3	8.7	8.7
					caco-2	>30	>30
					cem	2.9	2.9
1067	492.6888		MDA	0.070*	mda		
						>30	>30
			MDA	0.43			
1068	506.7567		MDA	> 30	mda		
						>30	>30
1069	459.431		mda	>1			
			MDA	0.74			

Fig. 9B (cont'd)

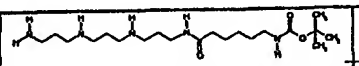
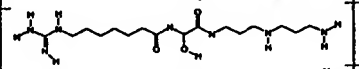
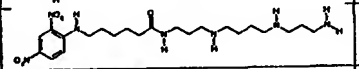
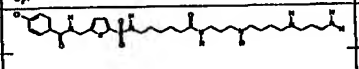
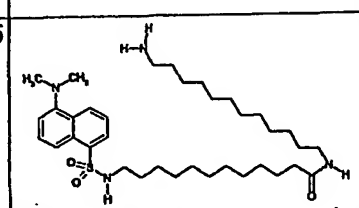
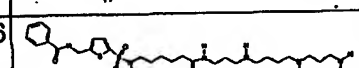
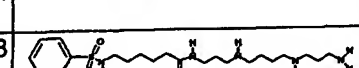
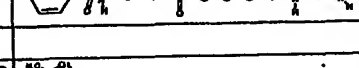
1083	401.5974				mda		>100
1085	373.5025		mda	81.3	mda		>300
1086	481.6		mda	2.2			
1090	629.2897		mda	0.0147	mda	0.960	300
			MDA	0.00997			
			PC-3	0.070*			
			MDA	0.01324			
			MCF-7	0.0252			
			CaCo	0.013*			
			MDA	0.022*			
			MDA	13.3 - 15.7 nM	mda	1.54	>300
			MDA	0.0216 Pre-			
			MDA	0.0273			
			HT-29	0.0812			
			Du145	0.016			
1093	630.9845		mda	>30			
1096	594.8446		MDA	0.094*	mda	26.5	190
			MDA	0.0397			
			MDA	0.117			
1097	455.6678		MDA	0.0817	mda	5.24	1200
					mda	5.52	1200
1098	590.8348		MDA	2.1	mda	263	>1000

Fig. 9B (cont'd)

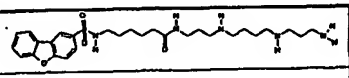
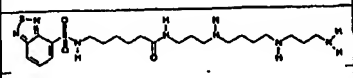
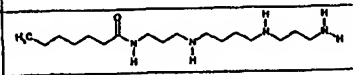
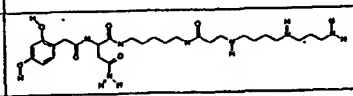
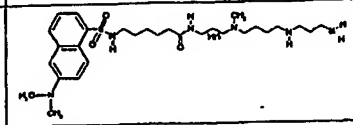
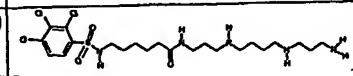
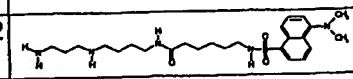
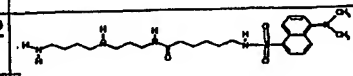
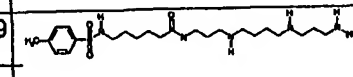
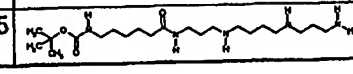
1100	545.75		MDA	0.0195*	mda	0.588	180
			MDA	0.00485			
			PC-3	0.0164			
			MDA	0.0105*			
			MCF-7	0.0196			
			CaCo	0.00663			
1101	513.7292		MDA	0.0793	pc-3	3.0	>300
					mda	6.17	>300
1107	314.5186		MDA	0.182	mda		63
1111	565.7189		MDA	0.19			
1113	564.8402		MDA	0.0167	mda	1.44	380
1114	559.0029		MDA	0.073	pc-3	1.43	320
					mda	1.59	>300
1115	491.7012						
					pc-3		>300
					mda	315	>300
1116	491.7012				pc-3		>300
					mda	315	>300
1119	469.6949		MDA	0.0568*	pc-3	5.1	>10
					mda	11.5	>10
1120	415.6245		MDA	0.0687*			

Fig. 9B (cont'd)

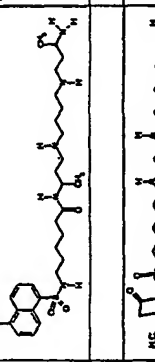
1122	343.5604		MDA	0.248					
			MDA	0.397					
1123	657.3438		MDA	0.012	MDA	5.20	255		
			MDA	0.0136	PC-3	1.23	530		
			PC-3	0.038					
			Du145	0.0985					
1124	576.8513		MDA	0.0178	mda	13.2	>300		
			MDA	0.0466					
1129	529.7915		MDA	0.17*	mda	68.2	>300		
					pc-3	71.3	>300		
1135	425.6633		MDA	0.167*	pc-3	29.2	>300		
					mda	66.5	>100		
1136	477.7398		MDA	0.0446*	mda	9.68	>1000		
			MDA	0.0344	pc-3	9.23	>1000		
1149	387.5703		MDA	0.136*	mda	>100	>100		
1152	590.8377		MDA	0.0903	pc-3		99		
			MDA	0.085	mda		>100		
1156	614.275		MDA	0.00955	mda	1.55	>300		
					pc-3	2.56	>300		
1160	393.5961		MDA	0.0564*	mda	45.8	>300		
					pc-3		64		
1161	357.5438		MDA	> 0.3	mda	>300	>300		
			MDA	> 1	pc-3	>300	>300		

Fig. 9B (cont'd)

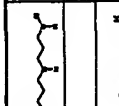
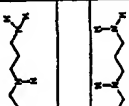
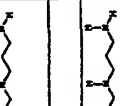
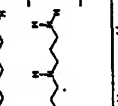
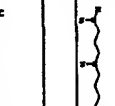
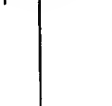
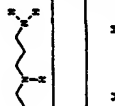
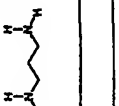
1165	607.2209		MDA	0.0143	mda	<3	199		
					pc-3	<3	188		
1174	459.66		MDA	0.3	mda	>300	>300		
					pc-3	>300	>300		
1175	373.5432		MDA	0.061	mda	>300	>300		
					pc-3	24.7	>300		
1179	369.555		MDA	> 1 uM	mda	>300	>300		
1180	439.6684		MDA	0.0265	mda	>300	>300		
					pc-3	>300	>300		
1203	244.3832								
1209	359.52		MDA	>1	mda	62	277		
					pc-3	72	227		
1233	587.2084		MDA	0.0355*	mda	1.9	>300		
			MDA	0.0185*	pc-3	0.56	>300		
1234	506.7159		MDA	0.0565	mda	1.6	>300		
					pc-3	0.87	>300		
1238	364.5792		MDA	> 1	mda		235		
					pc-3		208		
1239	392.6333				mda		195		
					pc-3		173		

Fig. 9B (cont'd)

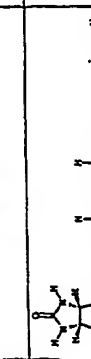
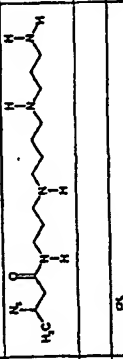
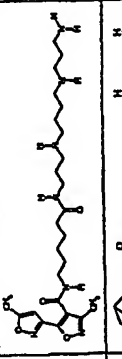
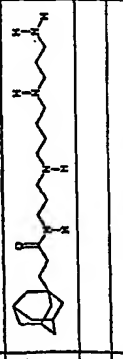
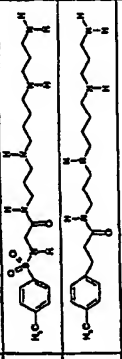
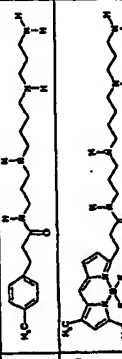
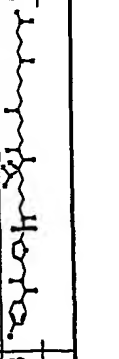
1241	615.2626		MDA	0.0262						
1243	428.6448									
1244	359.5189		MDA	0.48						
1245	313.4495									
1254	505.666		MDA	0.0577						
1281	392.6333		MDA	> 1						
1298	413.5865									
1305	348.5361									
1315	477.4338									
1340	644.3043									

Figure 9C

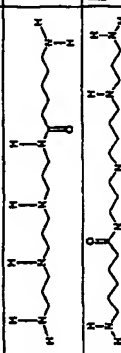
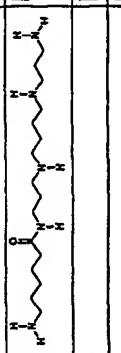
N1-monosubstituted polyamines: amides, amino alkyl							
ID	mol weight	Structure	Transport>Cell Line	Ki	Growth Inhibtion>Cell Line	Half Effect Drug DFMO	IC50
1091	301.4791				mda		>100
1094	315.5062		MDA	0.075	mda	18	>300
			MDA	0.117	mda	51.5	>1000
			MDA	0.040			
			MDA	0.028 -	mda	54	>300
			MDA	0.043			
1110	244.3832		MDA	0.162	MDA		
			MDA	0.190			
1121	343.5604		MDA	0.64	MDA	>300	>300
			MDA	0.5	PC-3		>300
1122	343.5604		MDA	0.248			
			MDA	0.397			
1126	301.4791		MDA	> 10	mda		>100
1150	287.452		MDA	0.043*	mda		>100
1177	273.4249		MDA	0.0756*	mda	>300	>300
			PC-3	0.0636	pc-3	<3	>300
			Du145	0.147	MDA	>100	>100
					PC-3	2.85	>100
1197	301.4791		MDA	0.39	MDA		>300
					PC-3	>300	460

Fig. 9C (cont'd)

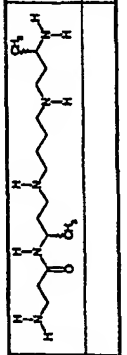
1198	301.4791		MDA		0.424	MDA	>300	>300
						PC-3	299	>300

Fig. 9D

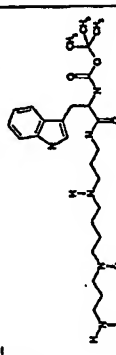
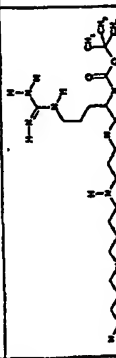
N1-monosubstituted polyamines: amides, protected amino acid head group									
ID	mol weight	Structure	Transport>Cell Line	Ki	Growth Inhibtion>Cell Line	Half Effect Drug DFMO	IC50		
1117	359.5161		MDA	0.232*	mda		>100		
1118	488.679				pc-3	22.64	>300		
					mda	50.4	>100		
1127	458.6526								
1147	481.7281		MDA	0.098*	mda	>100	>100		
1151	416.5685		MDA	> 1					
1153	430.5955		mda	0.156					
1155	401.5974		MDA	0.258					
1158	399.5815		MDA	0.183					
1162	433.6614		MDA	0.0913					
			MDA	0.083					
1170	521.7061		MDA	> 1	mda	>300	>300		

Fig. 9D (cont'd)

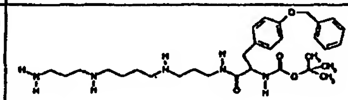
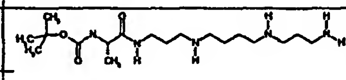
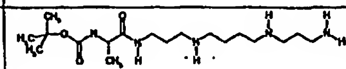
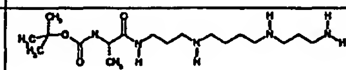
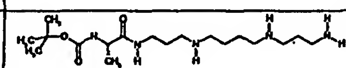
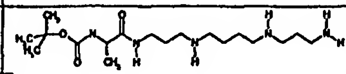
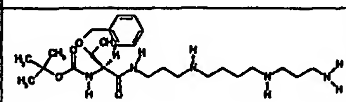
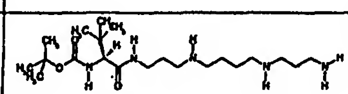
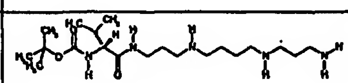
					pc-3	>300	>300
1172	555.7673		MDA	37.1	mda		20
					pc-3		20
1176	373.5432		MDA	0.0418	mda	>300	>300
			MDA		pc-3	14.0	>300
1176	373.5432		MDA	0.0418	mda	>300	>300
			MDA		pc-3	14.0	>300
1176	373.5432		MDA	0.0418	mda	>300	>300
			MDA		pc-3	14.0	>300
1176	373.5432		MDA	0.0418	mda	>300	>300
			MDA		pc-3	14.0	>300
1176	373.5432		MDA	0.0418	mda	>300	>300
			MDA		pc-3	14.0	>300
1189	493.6956		MDA	0.465	MDA	52	>300
					PC-3	100	>300
					pc-3	>300	>300
1193	415.6245		MDA	0.265	MDA	89.2	>300
					PC-3	91.9	>300
1195	401.5974		MDA	0.271	MDA	37.9	>300
					PC-3	70.9	>300

Fig. 9D (cont'd)

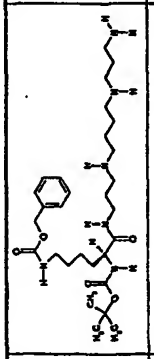
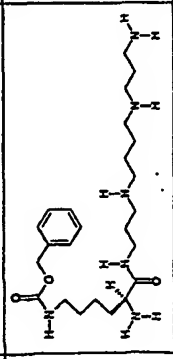
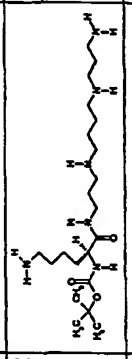
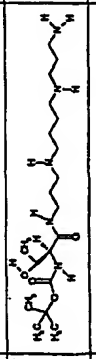
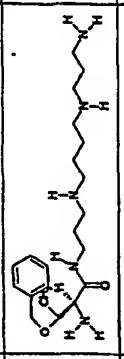
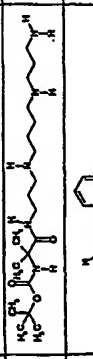
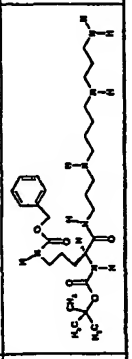
1199	564.775		MDA	0.060*	MDA	15.5	>300
					PC-3	9.20	>300
1200	464.6567		MDA	0.039	MDA	29.8	>300
					MDA	41.3	>300
					PC-3	7.87	>300
					PC-3	8.51	>300
1201	430.6392		MDA	0.191	MDA	36.9	>300
					PC-3	16.9	430
1205	403.5697				mda	100	>300
					pc-3	>300	>300
1206	393.5773		MDA	0.1094	mda	19	>300
					pc-3	67	>300
1219	387.5703						
1221	550.7479						

Fig. 9D (cont'd)

1222	450.6296				
1223	416.6121				
1229	415.6245				
1231	415.6245				
1259	760.9417				

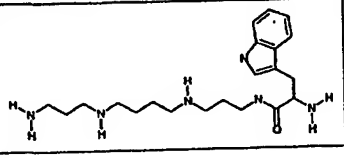
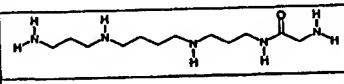
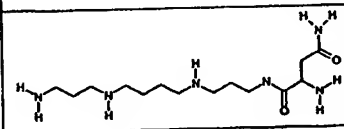
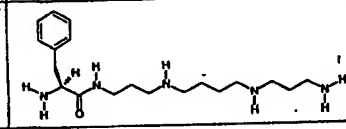
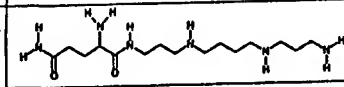
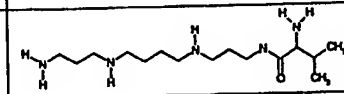
N1-monosubstituted polyamines: amides, natural alpha-amino acid head group							
ID	mol weight	Structure	Transport>Cell Line	Ki	Growth Inhibtion>Cell Line	Half Effect Drug-DFMO	IC50
1095	388.5607		MDA	0.073	mda	5.3	>300
					mda	8.44	560
			MDA	0.011 -	pc-3	14.05	>1000
					mda	30.0	>300
1125	259.3978		MDA	0.07	mda		>100
			MDA	0.1036*			
1131	316.4501		MDA	0.0325	pc-3	57.0	>300
					mda	81.97	>1000
					mda	113	>300
					pc-3	57	>300
1148	349.5237		MDA	0.214*	mda		>100
1154	330.4772		MDA	0.047	mda	>300	>300
					pc-3	>300	>300
1157	301.4791		MDA	0.160*	mda	5.58	>300
			MDA	0.0392	pc-3	14.35	>300
			PC-3	0.149	MDA	26.42	>300
			Du145	0.109	PC-3	3.86	>300
			MDA	0.0514	pc-3	5.28	
			Du145	0.0467			

Fig. 9E

Fig. 9E (cont'd)

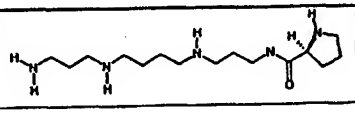
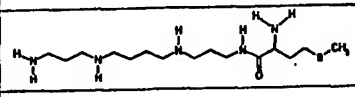
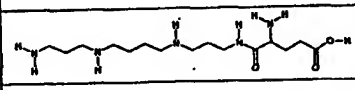
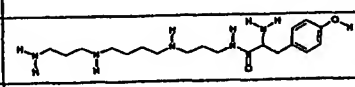
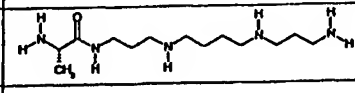
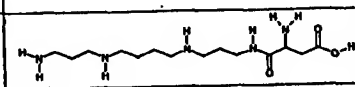
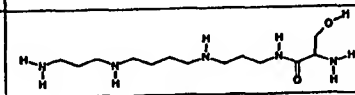
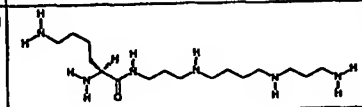
1159	299.4632		MDA	0.0255	mda	92.8	>300
			MDA	0.0499	pc-3	16.5	81
			MDA	21,5 - 50	mda	>100	>100
					pc-3	12.1	>100
1164	333.5431		MDA	0.0335	mda	>300	>300
					pc-3	>300	>300
1171	331.462		MDA	0.0765	MDA	300	>300
			MDA	0.13	PC-3	185	>300
1173	365.5231		MDA	0.0768	MDA	94.6	>300
					PC-3	42.7	>300
1178	273.4249		MDA	0.0526*	mda	>300	>300
					pc-3	>300	>300
1186	317.4349		MDA	0.167	MDA	300	>300
			MDA	0.38	PC-3	213	>300
1187	289.4243		MDA	0.0453	MDA	25.5	>300
					PC-3	20.8	>300
1202	330.5209		MDA	0.0295	MDA	4.75	>300
			PC3	0.748	PC-3	5.30	>300
			MDA	0.147	pc-3	1.7	
			MDA	0.032*			
			MDA	0.05			
			HT-29	0.185			

Fig. 9E (cont'd)

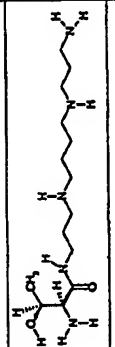
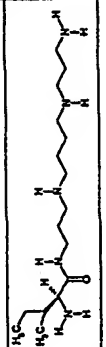
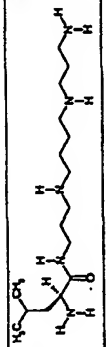
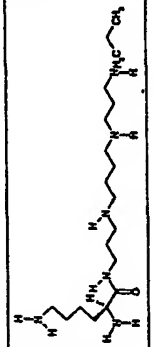
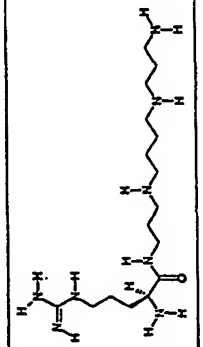
1207	303.4514		MDA	0.13	mda	6.5	>300			
					pc-3	62	>300			
1228	315.5062		MDA	0.124	mda	9.1	>300			
					pc-3	4.0	>300			
1230	315.5062		MDA	0.0323	mda	>300	>300			
					pc-3	6.2	>300			
1237	374.6181		MDA	0.113	mda	>300	>300			
					pc-3	>300	>300			
1260	358.5343		MDA	0.099	mda	6.80	>100			
					pc3	3.04	>100			

Fig. 9F

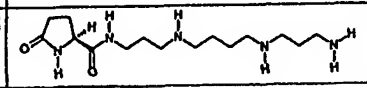
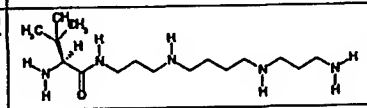
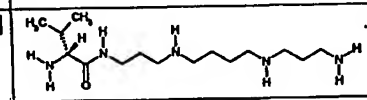
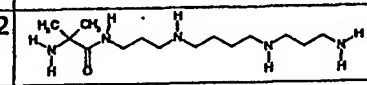
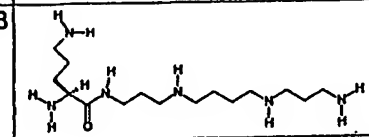
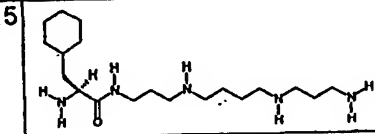
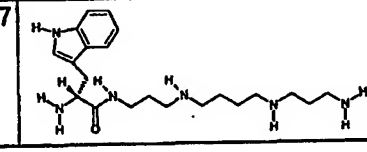
N1-monosubstituted polyamines: amides, non-natural alpha-amino acid head group							
ID	mol weight	Structure	Transport>Cell Line	Ki	Growth Inhibtion>Cell Line	Half Effect Drug DFMO	IC50
1188	313.4466		MDA	> 1 uM	MDA		320
			MDA	10.6	PC-3		214
1194	315.5062		MDA	0.0727*	MDA	5.32	>300
					PC-3	7.51	>300
					MDA	16.19	>300
					PC-3	1.82	>300
1196	301.4791		MDA	0.0483	MDA	9.03	>300
					PC-3	8.01	>300
1220	287.452		MDA	0.16	mda	8.0	>300
					pc-3	2.4	>300
1224	316.4938		MDA	0.0432	pc-3	3.0	>300
			MDA	0.0515	mda	4.37	>300
1227	355.5715		MDA	0.241	mda	7.8	>30
					pc-3	0.95	>30
1309	388.5607						

Fig. 9G

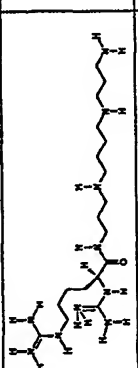
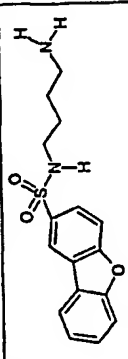
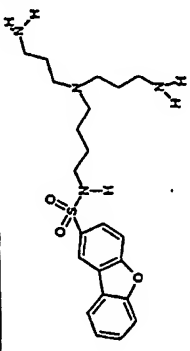
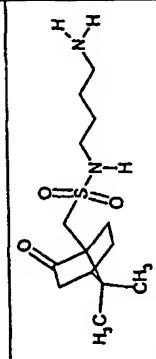
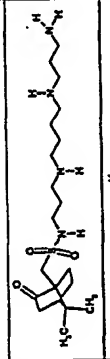
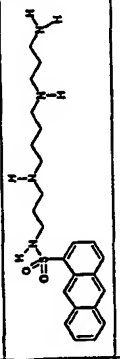
N1-monosubstituted polyamines: amides, amino acid derivative head group								
ID	mol weight	Structure	Transport>Cell Line	Ki	Growth Inhibtion>Cell Line	Half Effect Drug DFMO	IC50
1304	418.6337				mda	85	>300
					pc-3	15.0	244.8
1310	510.7726				mda	4.2	
					pc-3	1.7	
1355	145.206				mda		>10000

Fig. 9H

N1-monosubstituted polyamines: sulfonamides								
ID	mol weight	Structure	Transport>Cell Line	Ki	Growth Inhibtion>Cell Line	Half Effect Drug DFMO	IC50	
1001	435.6365		MDA	.039	MDA	20	600	
			A172	.08	A172			
1003	421.6094		MDA	1	MDA	100uM	>300	
							28 uM	
1005	318.3975		A172	23	A172			
							40 uM	
1006	446.6164		mda	1.46	A172		20	
							50	
1007	302.4389		A172	60	mda		>300	
1008	416.6308		MDA	>10	MDA		>300	
1010	442.6282		MDA	0.110	MDA	1.7	20	
			A172	0.082	MDA	1.05	18	

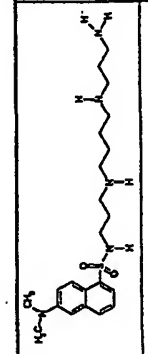
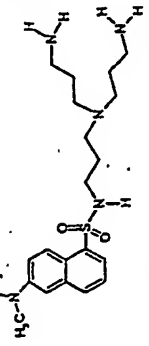
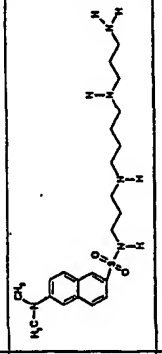
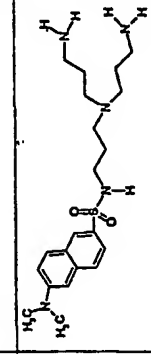
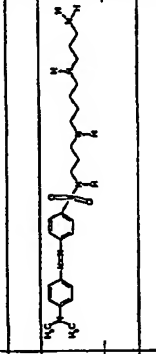
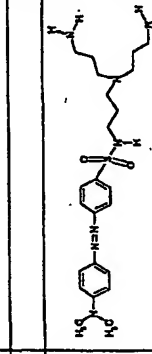
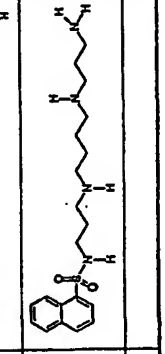
1011	435.6365		MDA	0.066*	MDA	6.0	50		
			A172	1.34	MDA	<3.0	50		
1012	421.6094		MDA	>10	MDA		150		
1013	435.6365		MDA	3.5	MDA	13.4	50		
			A172	1.34	MDA		100		
1014	421.6094		MDA	>10	MDA		100		
1015	489.6881		MDA	2.9	MDA		15		
			A172	1.6	pc-3		>30		
					caco-2		18.2		
					cem		>30		
1016	475.661		MDA	>10	MDA		13		
1017	392.5676		MDA	.187	MDA	14.2	50		
			A172	.24					

Fig. 9H (cont'd)

Fig. 9H (cont'd)

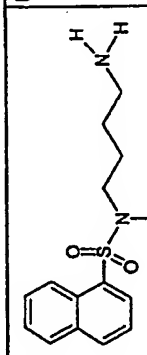
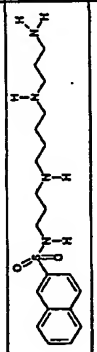
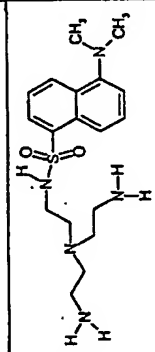
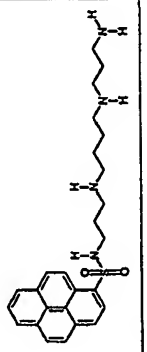
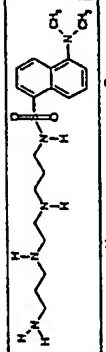
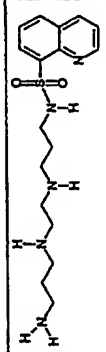
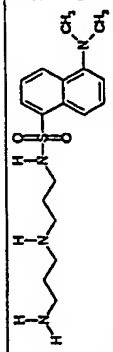
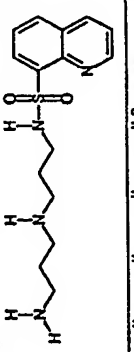
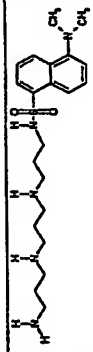
1018	278.3758		mda	>30	MDA				120
1019	392.5676		MDA	0.2*	MDA				50
			A172	0.37	MDA				50
1020	379.5281		MDA	>30	MDA				110
1023	466.6505		MDA	.091	MDA				22
			A172	.075					
1024	407.5823		MDA	5.4	MDA				50
1025	365.501		MDA	4.3	MDA				>300
1026	364.5135		MDA	2.7	MDA				50
1027	322.4322		MDA	>10	MDA				>300
1028	421.6094		MDA	11.4	MDA				50

Fig. 9H (cont'd)

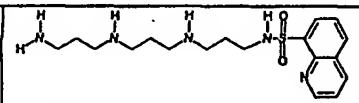
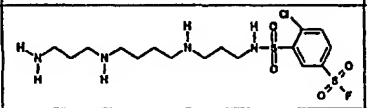
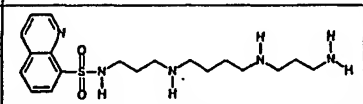
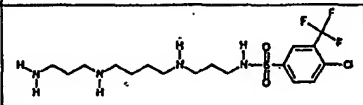
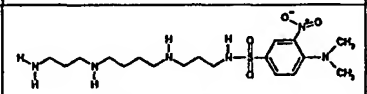
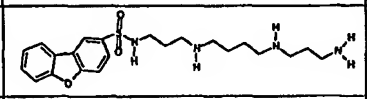
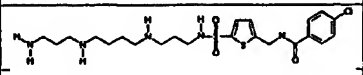
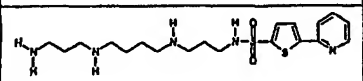
1029	379.5281		MDA	3.4	MDA		>300
1030	459.0054		MDA	0.08	MDA	125	>250
1031	393.5552		MDA	0.43	MDA	<10	>300
1034	444.9505		MDA	0.24	MDA	<3	50
					mda	8.7	50
1036	430.5735		MDA	0.84	MDA		>300
1041	432.5893		MDA	0.066	MDA	.95	12
					pc-3		6.2
					caco-2		16.1
					cem		0.79
					mda	12.6	53.0
					pc-3		12.4
					mda		46.1
					pc-3		6.5
1044	516.129		MDA	0.156*	MDA	3	180
			MDA	0.0582	mda	<3.0	190
			MDA	0.130			
			MDA	0.13			
1045	425.6192		MDA	0.228	MDA	13	180
			MDA	0.164	mda	7.3	140
			MDA	0.32			

Fig. 9H (cont'd)

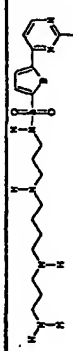
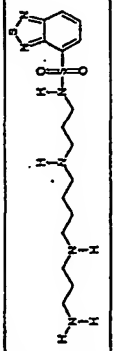
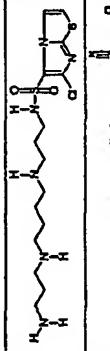
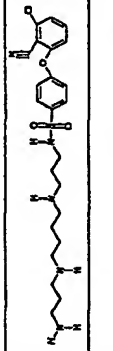
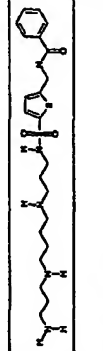
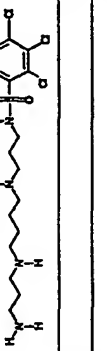
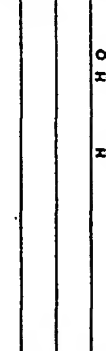
1046	472.6979		MDA	0.44	mda	6.92	58	
			MDA	0.0677	pc-3		34.8	
					caco-2		>30	
					cem		8.9	
1047	488.6944		MDA	0.375	mda	7.3	170	
			MDA	0.177				
1048	400.5686		MDA	0.421	mda	26.7	>300	
1049	423.0024		MDA	> 3	mda		>300	
1050	494.0602		MDA	0.108	MDA	2.26	140	
			MDA	0.0537				
1051	481.684		MDA	0.28	mda	6.5	>300	
			MDA	0.076				
1052	342.5071		MDA	0.16*	mda	30	>300	
1054	445.8422		MDA	0.025	MDA	<3.0	50	
			MDA	0.0829	mda	7.89	20	
					pc-3		19.8	
					caco-2		27.1	
					cem		2.6	
1057	434.7334		MDA	0.17	mda		100	

Fig. 9H (cont'd)

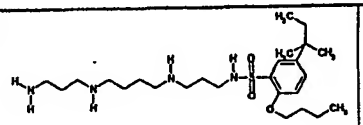
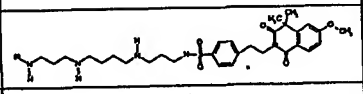
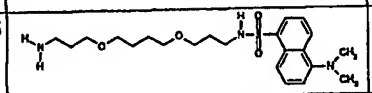
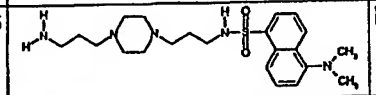
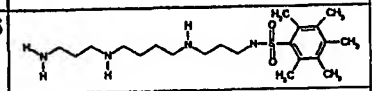
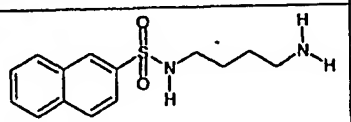
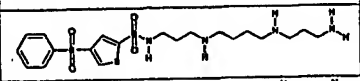
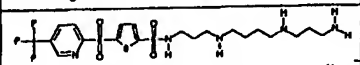
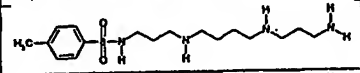
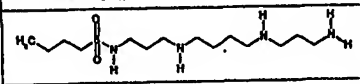
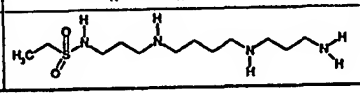
1058	484.7503		MDA	0.17*	mda		6
					pc-3		5.9
					caco-2		14.8
					cem		0.71
1070	587.7877		MDA	> 10			
					mda		13
					pc-3		>30
					caco-2		>30
					cem		>30
1074	437.606		MDA	> 30	MDA		
1075	433.6206		MDA	> 100			
1082	412.6426		MDA	> 3	mda		140
1088	278.3758		mda	5.4*			
1103	488.6944		MDA	0.067	mda	3.5	58
1105	557.6804		MDA	0.083	mda		44
1106	356.5342		MDA	0.094	mda		160
1108	322.5167		MDA	0.19	mda		150
1130	294.4625		MDA	0.22	mda	>300	>300

Fig. 9H (cont'd)

Fig. 9I

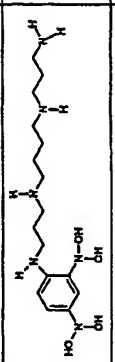
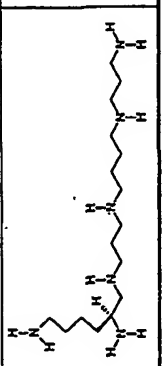
N1-monosubstituted polyamines: N1-monosubstituted amines								
ID	mol weight	Structure	Transport>Cell Line	Ki	Growth Inhibtion>Cell Line	Half Effect Drug DFMO	IC50	
1004	372.4712		MDA	2.2	MDA	5		
			A172	3				
1350	316.5374							

Fig. 9J

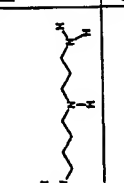
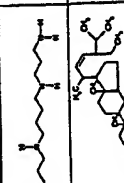
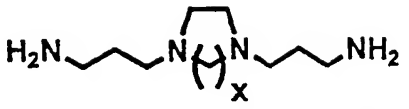
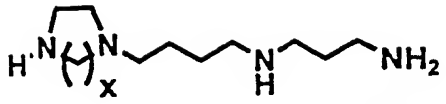
N1-monosubstituted polyamines: Other								
ID	mol weight	Structure	Transport>Cell Line	Ki	Growth Inhibition>Cell Line	Half Effect Drug DFMO	IC50
1021 (urea)	421.5906		MDA	0.44	MDA	8.2	35
			A172	.04*			
1042 (urea)	569.7752		MDA	1	MDA	14.8	100
1071	641.0454		MDA				
1109 (urea)	563.8118		MDA	0.0674	pc-3	30	>100
			MDA	0.090	mda	95	>100
1295 (thiourea)	591.735		MDA	>3			

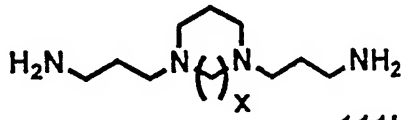
Fig. 10



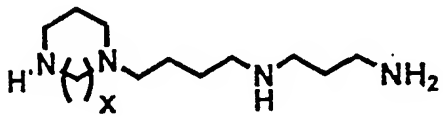
111a



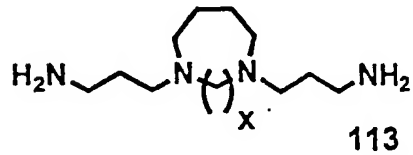
112a



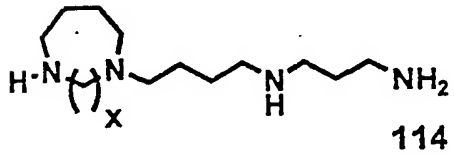
111b



112b

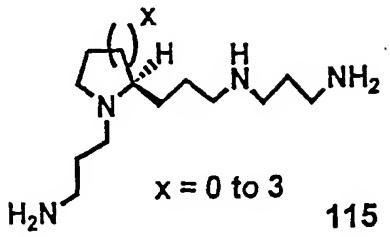


113



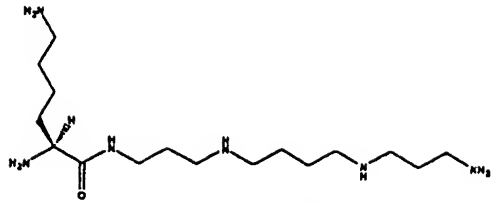
114

x = 1 to 4

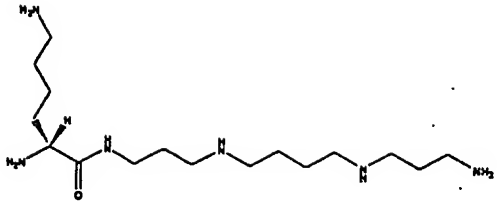


x = 0 to 3

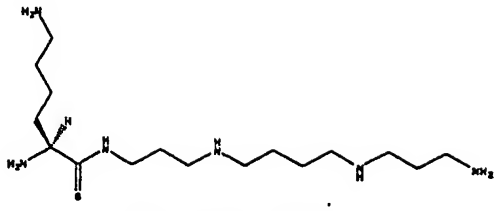
115



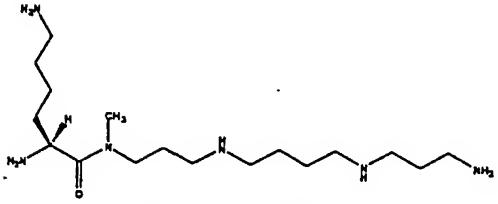
Compound 1202
L-Lys-Spm



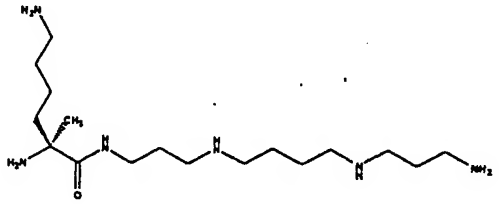
Compound 1390
D-Lys-Spm



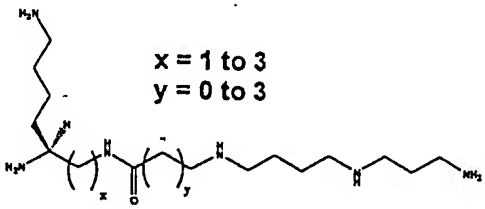
Compound 1380
L-Lys-Spm Thioamide



Compound 1391
L-Lys-Spm (methylamide)



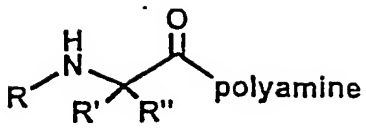
Compound1392
L-Lys-Spm (α-methyl)



x = 1 to 3
y = 0 to 3

Compound 1393 - 1405
L-Lys-Spm (isoamide)

Fig. 11



stereochemistry:
L is S, D is R

R'		R'	
-H	Gly	HS	Cys
$-CH_3$	Ala	S	Met
	Val	H_2N O	Asn
	Leu	H_2N O	Gln
	Ile	HO O	Asp
	Phe	HO O	Glu
HO	Tyr	H_2N	Lys
N H	Trp	H_2N	Orn
HO	Ser	H_2N H N NH	Arg
OH	Thr	N N H	His
		N H OH O	Pro

Fig. 12

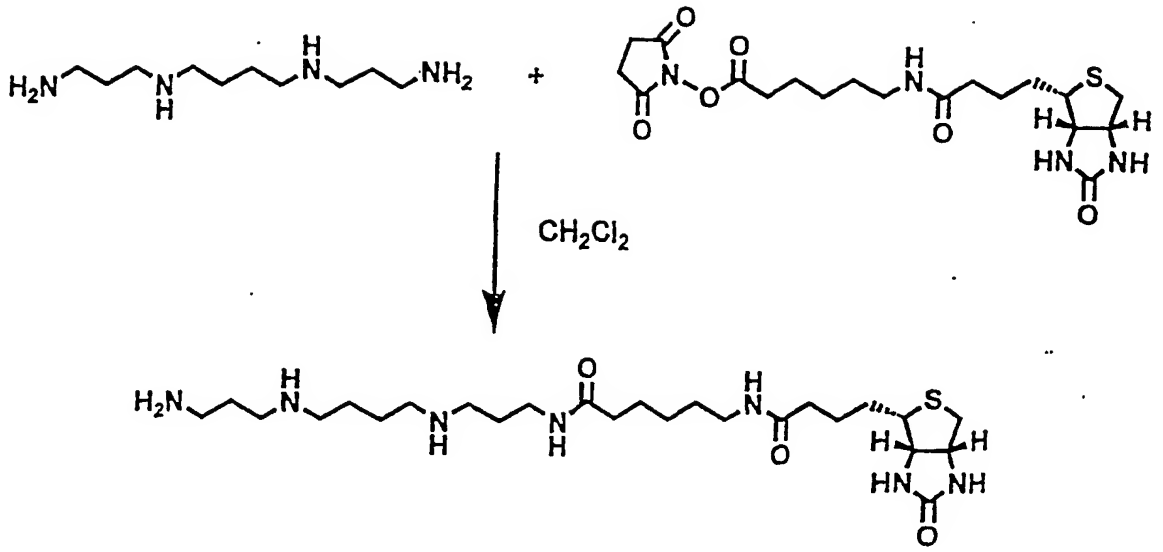


Fig. 13

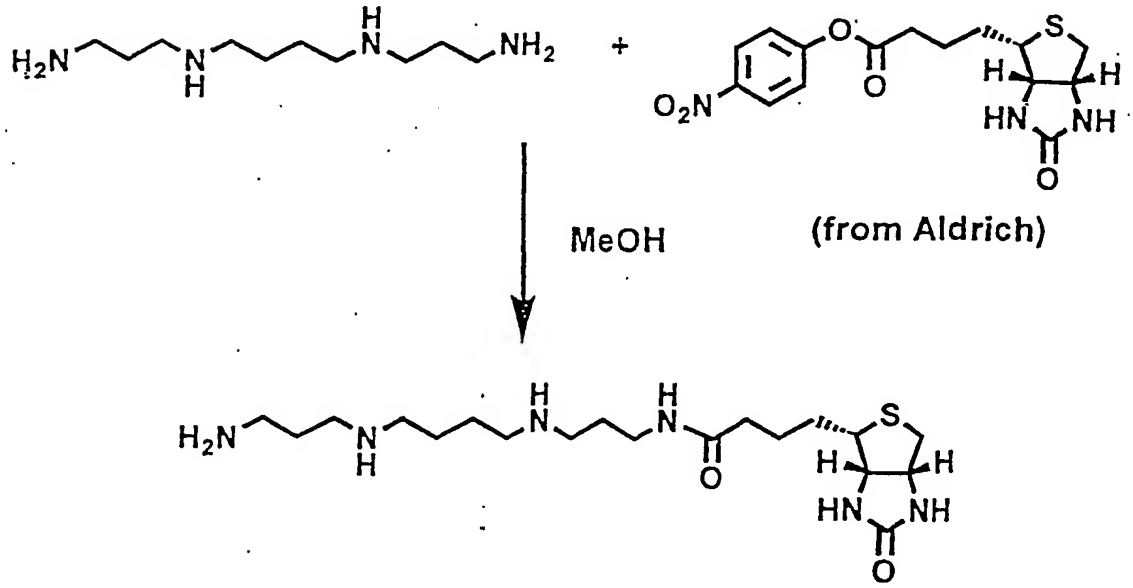


Fig. 14

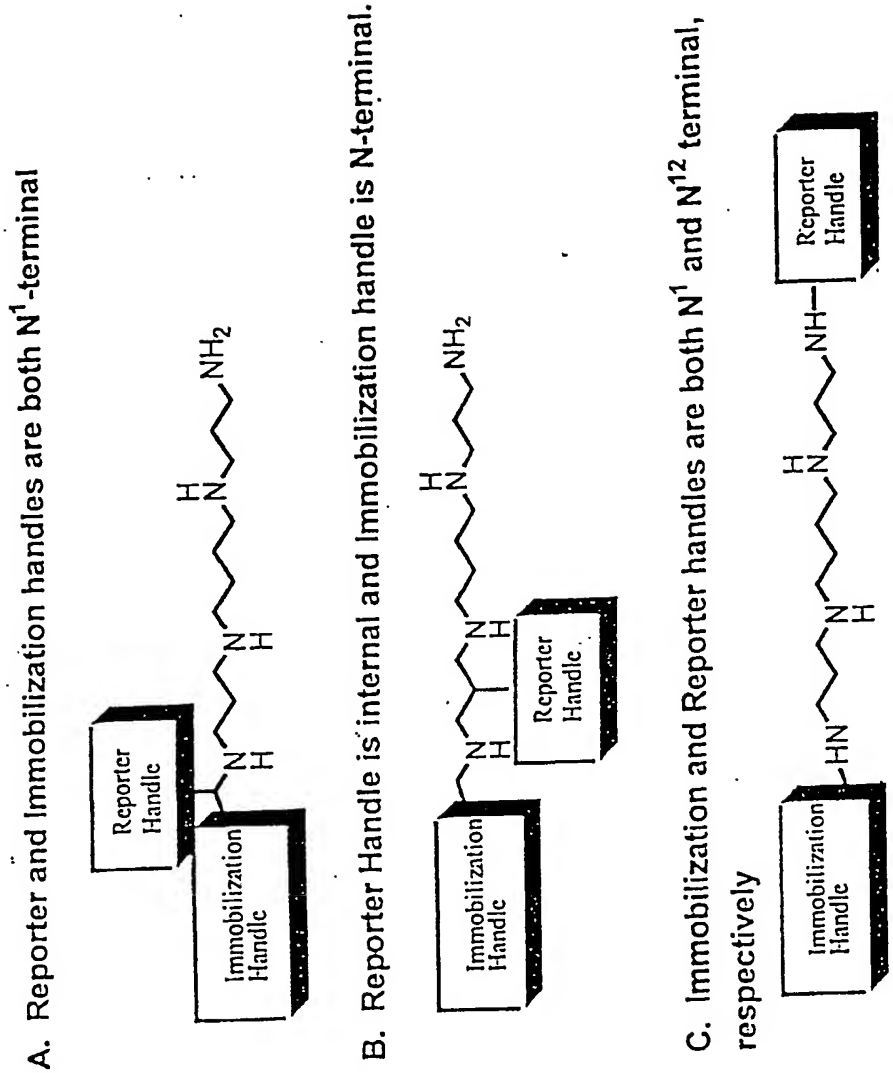

A. Reporter and Immobilization handles are both N1-terminal
Reporter Handle
Immobilization Handle
NH2
B. Reporter Handle is internal and Immobilization handle is N-terminal.
Immobilization Handle
Reporter Handle
NH2
C. Immobilization and Reporter handles are both N1 and N12 terminal, respectively
Immobilization Handle
Reporter Handle
NH

Fig. 15

Bispolyamine derivatives: deprotected

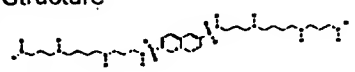
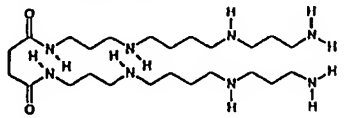
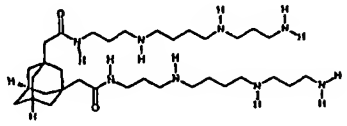
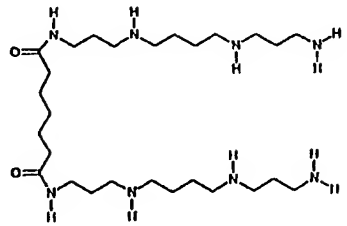
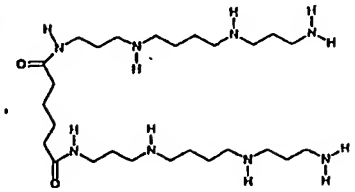
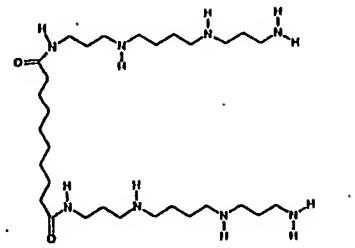
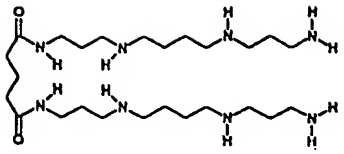
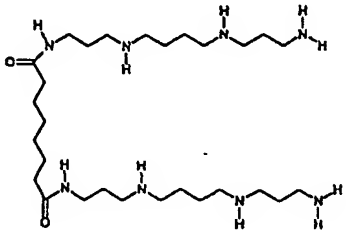
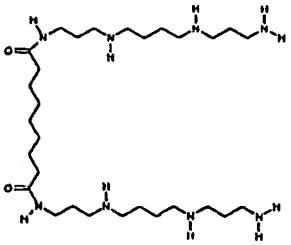
ID	mol weight	Structure	Transport>Cell Line	Ki	Growth Inhibtion>Cell Line	Half Effect Drug DFMO	IC50
1092	656.96		MDA	0.079	mda	279	>300
1236	486.7504		MDA	0.0288	mda	6.0	>300
			MDA	0.0152	pc-3	1.8	>300
1261	620.9735		MDA	0.402			
1275	528.8317		MDA	0.219			
1286	514.8046		MDA	0.0595			
1287	570.9129		MDA	0.0986			

Fig. 16

1288 500.7775 

1289 542.8587 

1290 556.8858 

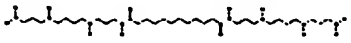
1299 598.9671 

Fig. 16 (cont'd)

Bispolyamine derivatives: protected

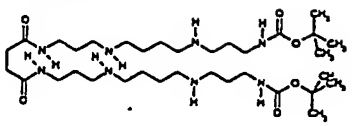
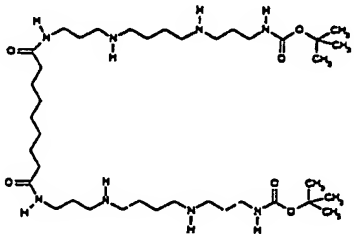
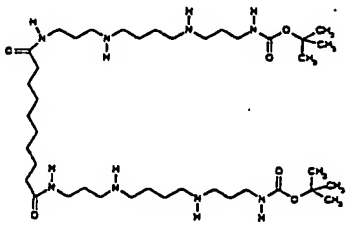
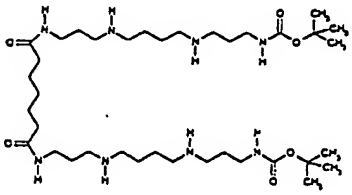
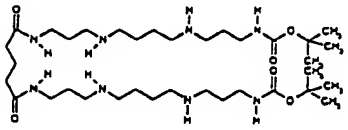
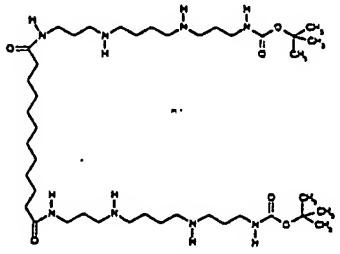
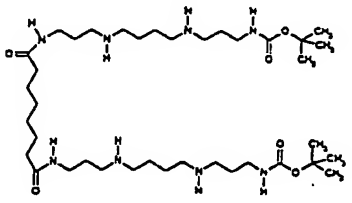
ID	mol weight	Structure
1262	686.987	
1263	757.1225	
1264	771.1495	
1265	729.0683	
1267	701.0141	
1268	799.2037	
1269	743.0954	

Fig. 17

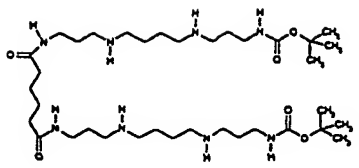
1285 715.0412 

Fig. 17 (cont'd)

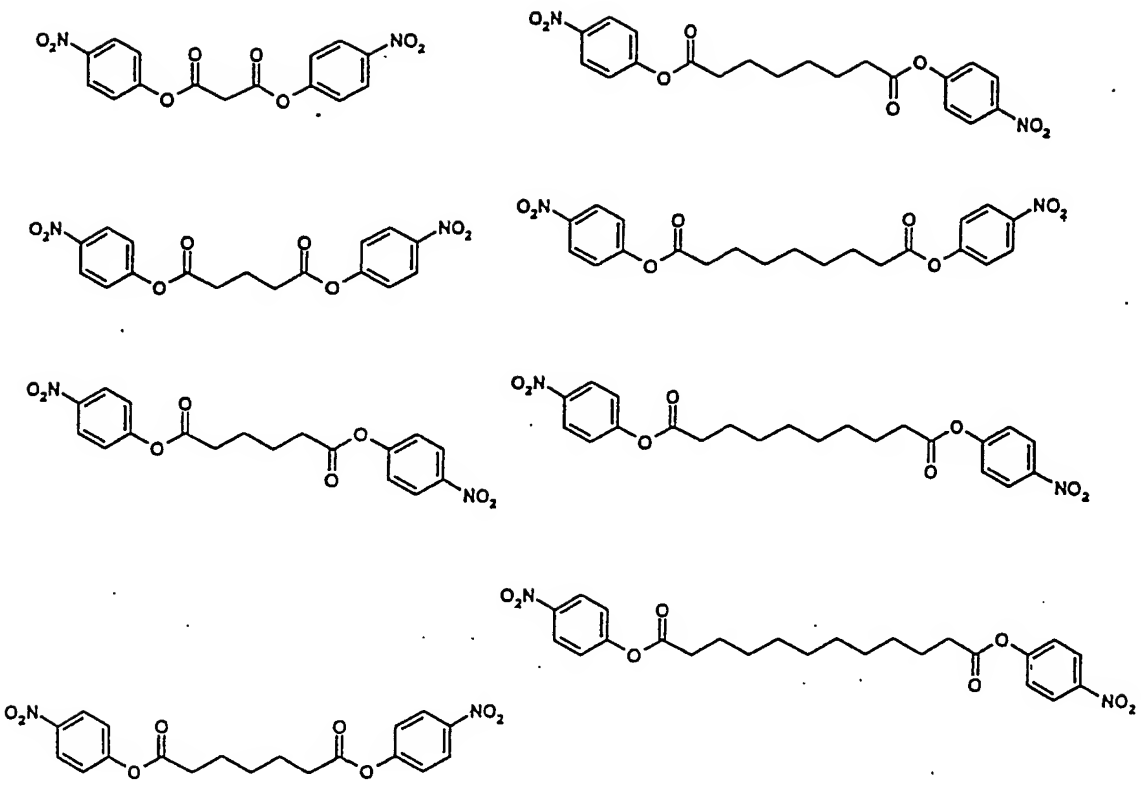


Fig. 18

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 December 2001 (06.12.2001)

PCT

(10) International Publication Number
WO 01/092218 A3

(51) International Patent Classification[7]: C07C 311/18, 233/36, 233/37, 271/10, 311/41, 311/11, C07D 307/77, 333/34, 409/04, A61K 31/165, 31/18, 31/33

(21) International Application Number: PCT/US01/17795

(22) International Filing Date: 31 May 2001 (31.05.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/584,175 31 May 2000 (31.05.2000) US

(71) Applicant: **ORIDIGM CORPORATION** [US/US]; Suite 200, 4010 Stone Way North, Seattle, WA 98103 (US).

(72) Inventors: **VERMEULIN, Nicolaas, M., J.**; 19334 - 196th Avenue NE, Woodinville, WA 98072 (US). **O'DAY, Christine, L.**; 4404-B 216th Street S.W., Mountlake Terrace, WA 98043 (US). **WEBB, Heather, K.**; 5705 Seaview Avenue, NW, Seattle, WA 98107 (US). **BURNS, Mark, R.**; 226 Northwest 184th Street, Shoreline, WA 98177 (US). **BERGSTROM, Donald, E.**; 3416 Hamilton Street, West Lafayette, IN 47906 (US).

(74) Agents: **LAU, Kawai** et al.; Morrison & Foerster LLP, Suite 500, 3811 Valley Centre Drive, San Diego, CA 92130-2332 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
27 March 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/092218 A3

(54) Title: POLYAMINE ANALOGUES AS THERAPEUTIC AND DIAGNOSTIC AGENTS

(57) Abstract: Novel "bispolyamine" inhibitor compounds of polyamine transport are disclosed. These compounds are useful pharmaceutical agents for treating diseases where it is desired to inhibit polyamine transport or other polyamine binding proteins, for example cancer and post-angioplasty injury. These compounds display desirable activities both for diagnostic and research assays and therapy.

INTERNATIONAL SEARCH REPORT

ational Application No
PCT/US 01/17795

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07C311/18 C07C233/36 C07C233/37 C07C271/10 C07C311/41
C07C311/11 C07D307/77 C07D333/34 C07D409/04 A61K31/165
A61K31/18 A61K31/33

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07C C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 03823 A (ORIDIGM CORP) 28 January 1999 (1999-01-28) page 20, line 29 - page 21, line 11; page 43, line 25 - page 46, line 27; page 58, line 21 - page 62, line 14; example VIII; figure 2, compound 42 & PCT/US98/14896 cited in the application	1,10, 16-30
X	WO 99 54283 A (UNIVERSITE LAVAL ET AL) 28 October 1999 (1999-10-28) page 7, line 14 - page 8, line 2; claims 1-12, 15-18, 21-43	1,19,20, 23,26,27
X	G.J. ATWELL ET AL: J. MED. CHEM. , vol. 29, no. 1, 1986, pages 69-74, XP000891842 the whole document	1-3,6, 23-25

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 December 2002	03/01/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Van Amsterdam, L

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

ıational Application No
PCT/US 01/17795

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	G. SOSNOVSKY ET AL: Z. NATURFORSCH., B: CHEM. SCI., vol. 49, no. 11, 1994, pages 1580-1585, XP001040443 pages 1580-1581, introduction; page 1581, scheme 2, compounds 10-11, 13-14; page 1582, scheme 4, compounds 20-21 ---	1-3,23
X	C. Q. XIA ET AL: J. DRUG TARGETING , vol. 6, no. 1, 1998, pages 65-77, XP001040438 page 71, compounds 31, 39, 41, 34, 38, 40, 44; page 73, compounds 60, 61 ---	1,2,23
X	M. K.-H. DOLL ET AL: HELV. CHIM. ACTA, vol. 79, no. 2, 1996, pages 541-547, XP001040442 page 543, scheme 2, compounds 8-12 ---	1-3
P,X	EP 1 085 011 A (ORIDIGM CORP) 21 March 2001 (2001-03-21) page 10, lines 6-26; page 16, lines 47-49; page 23, line 54 - page 25, line 15; example VII; figure 2/5, compound 42; figures 44b-c -----	1-4,6, 10,19-30

INTERNATIONAL SEARCH REPORT

ternational application No.
PCT/US 01/17795

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 23-30 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Continuation of Box I.2

Present claim 1 relates to bispolyamine compounds defined by reference to a desirable property, namely the ability of these compounds to bind to a polyamine-binding site of a molecule and/or to inhibit polyamine transport. The claim cover all bispolyamine compounds having this property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a limited number of such bispolamine compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the bispolamine compounds by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been guided those parts of the application which do appear to be sufficiently clear. The search has mainly related to bispolyamine compounds consisting of two, optionally N-monosubstituted, polyamine moieties selected from putrescine, spermidine and spermine (cf. claim 2), linked together via a linking group derived from a dicarboxylic or disulfonic acid (cf. figures 16-17).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

tional Application No
PCT/US 01/17795

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9903823 A	28-01-1999	AU 8496898 A	10-02-1999
		EP 1001927 A2	24-05-2000
		JP 2001510181 T	31-07-2001
		WO 9903823 A2	28-01-1999
		US 6172261 B1	09-01-2001
WO 9954283 A	28-10-1999	WO 9954283 A1	28-10-1999
		AU 7131698 A	08-11-1999
		CA 2304557 A1	28-10-1999
		EP 1003715 A1	31-05-2000
EP 1085011 A	21-03-2001	AU 5942100 A	22-03-2001
		EP 1085011 A1	21-03-2001
		JP 2001172244 A	26-06-2001